DAC

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April 11, 2010

Commissioner for Patents
U.S. Patent and Trademark Office
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Mail Stop Patent Ext.

Re: U.S. Patent No. 7,008,765 B1; Issue Date: March 7, 2006
For: **PCA3, PCA3 Genes, and Methods of Use**
Inventors: Bussemakers *et al.*
Our Ref: 2218.034STR0/ELE/HCC

Sir:

Transmitted herewith for appropriate action are the following documents:

1. Credit Card Payment Form (PTO-2038) in the amount of $1,120.00 to cover fee for Application for Extension of Patent Term Pursuant to 35 U.S.C. §156;

2. Application for Extension of Patent Term Pursuant to 35 U.S.C. §156, signed by R. William Bowen, Jr., of Gen-Probe Incorporated, agent for The Johns Hopkins University and Stichting Katholieke Universiteit, more particularly the University Medical Centre Nijmegen *(in triplicate)*, and Exhibits 1-18 *(each in triplicate)*; and

3. One (1) return postcard.

It is respectfully requested that the attached postcard be stamped with the date of filing of these documents, and that it be returned to our courier.

In the event that extensions of time are necessary to prevent abandonment of this Application for Extension of Patent Term, then such extensions of time are hereby petitioned.

04/12/2012 LHGUYEN1 00000032 7008765
01 FC:1457 1120.00 OP

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

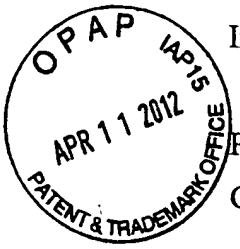
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Helene C. Carlson
Attorney for Gen-Probe Incorporated, Agent for Applicants
Registration No. 47,473

ELE/HCC/mik
Enclosures

1511652_1.DOC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re United States Patent of:
Bussemakers and Isaacs
Patent No.: 7,008,765 B1
Granted: March 7, 2006

Atty. Docket: 2218.034STR0/HCC/ELE

For: PCA3, PCA3 Genes, and Methods of Use

APPLICATION FOR EXTENSION OF PATENT TERM PURSUANT TO 35 U.S.C. § 156

Mail Stop Patent Ext.

Commissioner for Patents
U.S. Patent and Trademark Office
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir:

Pursuant to 35 U.S.C. § 156 and 37 C.F.R. §§ 1.710-1.791, The Johns Hopkins University (Baltimore, MD) ("TJHU") and Stichting Katholieke Universiteit, more particularly the University Medical Centre Nijmegen (Nijmegen, NL) ("SKU-UMCN") (collectively, "Applicants") each represents that it is an owner and assignee of the entire interest in and to Letters Patent of the United States No. 7,008,765 B1 ("the '765 patent"; **Exhibit 1**), granted to Marion J.G. Bussemakers and William B. Isaacs, on March 7, 2006, for "PCA3, PCA3 Genes, and Methods of Use" by virtue of an assignment from inventor Bussemakers to University Hospital Nijmegen, recorded June 13, 2000, at Reel 010902, Frame 0386 (**Exhibit 4**), and change of name to University Medical Centre Nijmegen, recorded August 23, 2004 at Reel 015020, Frame 0591 (**Exhibit 5**), and corrective assignment to correct the spelling of the city in the receiving party section, recorded August 31, 2004, at Reel 015060, Frame 0902 (**Exhibit 6**), and corrective assignment to correct the name of the assignee to Stichting Katholieke Universiteit, more

particularly the University Medical Centre Nijmegen, recorded January 6, 2006, at Reel 017365, Frame 0965 (**Exhibit 7**), and by virtue of an assignment from inventor Isaacs to The Johns Hopkins University recorded December 23, 2004, at Reel 016106, Frame 0901 (**Exhibit 8**).

The '765 patent issued out of United States Patent Application No. 09/402,713, which is a national phase application of International Application No. PCT/CA98/00346, having an international filing date of April 9, 1998, which claims priority to United States Provisional Patent Application No. 60/041,836, filed April 10, 1997.

The approved product that is relevant to this application is PROGENSA® PCA3 Assay, referred to herein as "PROGENSA" or "Approved Product."

The Marketing Applicant for PROGENSA is Gen-Probe Incorporated. A letter on behalf of the Marketing Applicant authorizing the patent owners to rely upon the activities of the Marketing Applicant is attached hereto as **Exhibit 9**.

The following information is submitted by Gen-Probe Incorporated, through its duly authorized attorneys, Sterne, Kessler, Goldstein & Fox PLLC, on behalf of the Applicants (see **Exhibits 10 and 11**), in accordance with 35 U.S.C. § 156(d) and the rules for extension of patent term issued by the USPTO at 37 C.F.R. Subpart F, §§ 1.710-1.791 and follows the numerical format set forth in 37 § C.F.R. 1.740.

1. A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics;

The Approved Product is a diagnostic assay for determining the need for repeat biopsies in cases of suspected prostate cancer. The Approved Product is indicated for:

> The PROGENSA PCA3 Assay is an *in vitro* nucleic acid amplification test. The assay measures the concentration of prostate cancer gene 3 (PCA3) and prostate-specific antigen (PSA) RNA

> molecules and calculates the ratio of PCA3 RNA molecules to PSA RNA molecules (PCA3 Score) in post-digital rectal exam (DRE) first catch male urine specimens. The PROGENSA PCA3 Assay is indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PROGENSA PCA3 Assay results.
>
> A PCA3 Score <25 is associated with a decreased likelihood of a positive biopsy. Prostatic biopsy is required for diagnosis of cancer.

See current draft of label (**Exhibit 12**), p. 2; and FDA approval letter (**Exhibit 14**), p. 1.

PCA3 is a prostate-specific RNA that is highly over-expressed (60-100 fold) in prostate cancer cells over normal cells. This is in contrast to prostate-specific antigen (PSA), which is expressed at similar levels in cancerous and benign prostate cells. Therefore, elevated expression of PCA3 is much more specific for cancer than elevated serum PSA, and use of the Approved Product helps avoid the need to biopsy men with elevated serum PSA who do not have prostate cancer. *See* current draft of label (**Exhibit 12**), p. 3; and Physician Brochure (**Exhibit 13**), p. 2, col. 1.

The Approved Product is a highly specific urine-based assay. The assay quantitates PCA3 expression using a combination of target capture, Transcription Mediated Amplification ("TMA"), and Hybridization Protection Assay ("HPA"). To quantitate PCA3 expression, a laboratory isolates PCA3 target RNA molecules from prostate cells in urine specimens using capture oligonucleotides that are complementary to sequence-specific regions of the target RNA. The laboratory hybridizes the capture oligonucleotides to the target RNA and captures the hybridized target RNA onto magnetic particles that are then separated from the urine specimen in a magnetic field. The captured target RNA is washed and amplified using TMA. The TMA reaction is

performed using reverse transcriptase ("RT") and T7 RNA polymerase and a set of primers specific for PCA3. RT produces a DNA copy of the target PCA3 RNA that contains an added T7 promoter. T7 RNA polymerase produces multiple copies of PCA3 RNA amplicon from the DNA copy. The amplicon is detected using HPA and a labeled, single-stranded PCA3 nucleic acid probe that is complementary to the PCA3 amplicon and that hybridizes specifically to it. During HPA, the labeled PCA3 probe is hybridized to the PCA3 amplicon, a selection reagent inactivates the label on any unhybridized probe, and the label on the hybridized probe is measured. *See* current draft of label (**Exhibit 12**), p. 4; and Physician Brochure (**Exhibit 13**), p. 2, cols. 1-2.

The laboratory carries out a similar set of reactions in a separate tube to measure PSA RNA in prostate cells in the urine specimen using a set of oligonucleotides specific for PSA. The PCA3 Score is determined based on the ratio of PCA3 RNA to PSA RNA multiplied by 1000. As quoted above, "[a] PCA3 Score <25 is associated with a decreased likelihood of a positive biopsy." Current draft of label (**Exhibit 12**), p. 2; and Physician Brochure (**Exhibit 13**), p. 2, para. bridging cols. 1-2.

The Approved Product comprises several isolated nucleic acid molecules (referred to above as oligonucleotides, primers, and probes). The sequence information regarding these molecules is proprietary information of the Marketing Applicant. However, Applicants supply non-proprietary information below regarding these molecules to fulfill the requirements of 35 U.S.C. § 156.

The Approved Product contains at least one each of the following.

1. An isolated nucleic acid molecule comprising a 10-50 nucleotide probe or primer that hybridizes at 68°C in 5xSSC, 5x Denhardt's solution, 1% SDS, and 100 μg/ml salmon sperm DNA to at least one of SEQ ID NOs:1, 3, 4, or 6 of the '765 patent but that does not hybridize to nucleotides 511-985 of SEQ ID NO:1,

nucleotides 346-820 of SEQ ID NO:3, nucleotides 346-820 of SEQ ID NO:4, or nucleotides 533-1007 of SEQ ID NO:6, and that selectively hybridizes to polynucleotides over-expressed in prostate cancer tissue as compared to normal human tissues selected from the group consisting of: artery, brain, breast, duodenum, heart, liver, ovary, placenta, seminal vesicles, skeletal muscle, skin, spinal cord, spleen and testis.

2. An isolated nucleic acid molecule that consists of 10-50 nucleotides and that specifically hybridizes to PCA3 RNA or DNA, and that is, or is complementary to, a nucleotide sequence consisting of at least 10 consecutive nucleotides from PCA3 exon 1 (1-98 of SEQ ID NO:1 or 1-120 of SEQ ID NO:6), 2 (99-263 of SEQ ID NO:1 or 121-285 of SEQ ID NO:6), 3 (264-446 of SEQ ID NO:1 or 286-468 of SEQ ID NO:6), 4a (447-985 of SEQ ID NO:1 or 469-1007 of SEQ ID NO:6), 4b (986-2037 of SEQ ID NO:1 or 1008-2066 of SEQ ID NO:6), 4c (2067-2622 of SEQ ID NO:6), or 4d (2623-3582 of SEQ ID NO:6), and that does not specifically hybridize to nucleotides 511-985 of SEQ ID NO:1 or nucleotides 533-1007 of SEQ ID NO:6.

2. A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred;

The Approved Product is a medical device and the submission was approved under Section 515 of the Federal Food, Drug and Cosmetic Act ("FFDCA"), 21 U.S.C. § 360e.

3. An identification of the date on which the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred;

The Approved Product received permission for commercial marketing or use under Section 515 of the FFDCA, 21 U.S.C. § 360e, on February 13, 2012. A copy of the FDA approval letter is attached as **Exhibit 14**.

4. In the case of a drug product, an identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved.

The Approved Product is not a drug product; thus, this section is not applicable.

5. **A statement that the application is being submitted within the sixty day period permitted for submission pursuant to §1.720(f) and an identification of the date of the last day on which the application could be submitted;**

PROGENSA was approved on February 13, 2012, and the last day within the sixty day period permitted for submission of an application for patent term extension is April 12, 2012, which is subsequent to the date on which this application has been submitted.

6. **A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, the date of issue, and the date of expiration;**

Inventors:	Marion J.G. Bussemakers and William B. Isaacs
Patent number:	7,008,765 B1
Date of issue:	March 7, 2006
Date of expiration:	April 9, 2018

7. **A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings;**

A full copy of U.S. Patent No. 7,008,765, for which extension is being sought, is attached as **Exhibit 1**.

8. **A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent;**

A copy of a Certificate of Correction dated March 25, 2008, is attached as **Exhibit 2**. A statement showing maintenance fee payment for fee year 4 is attached as **Exhibit 3**. Maintenance fee payments for fee years 8 and 12 are not yet due.

9. A statement that the patent claims the approved product, or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which at least one such patent claim reads on the approved product, or a method of using or manufacturing the approved product:

Claims 1, 6, 8, 25, 27, 29, 31, 37 and 39 of the '765 patent read on the Approved Product, as detailed below for claim 6. Claims 7 and 30 read on the approved method of using the Approved Product, as detailed below for claim 7.

Claim 6 recites: An isolated nucleic acid molecule consisting of 10 to 50 nucleotides which specifically hybridizes to PCA3 RNA or DNA, wherein said nucleic acid molecule is, or is complementary to, a nucleotide sequence consisting of at least 10 consecutive nucleotides from PCA3 exon 1 (1-98 of SEQ ID NO:1 or 1-120 of SEQ ID NO:6), 2 (99-263 of SEQ ID NO:1 or 121-285 of SEQ ID NO:6), 3 (264-446 of SEQ ID NO:1 or 286-468 of SEQ ID NO:6), 4a (447-985 of SEQ ID NO:1 or 469-1007 of SEQ ID NO:6), 4b (986-2037 of SEQ ID NO:1 or 1008-2066 of SEQ ID NO:6), 4c (2067-2622 of SEQ ID NO:6), or 4d (2623-3582 of SEQ ID NO:6), and wherein said nucleic acid molecule does not specifically hybridize to nucleotides 511-985 of SEQ ID NO:1 or nucleotides 533-1007 of SEQ ID NO:6.

The Approved Product contains an isolated nucleic acid molecule consisting of 10 to 50 nucleotides which specifically hybridizes to PCA3 RNA or DNA, wherein said nucleic acid molecule is, or is complementary to, a nucleotide sequence consisting of at least 10 consecutive nucleotides from PCA3 exon 1 (1-98 of SEQ ID NO:1 or 1-120 of SEQ ID NO:6), 2 (99-263 of SEQ ID NO:1 or 121-285 of SEQ ID NO:6), 3 (264-446 of SEQ ID NO:1 or 286-468 of SEQ ID NO:6), 4a (447-985 of SEQ ID NO:1 or 469-1007 of SEQ ID NO:6), 4b (986-2037 of SEQ ID NO:1 or 1008-2066 of SEQ ID NO:6), 4c

(2067-2622 of SEQ ID NO:6), or 4d (2623-3582 of SEQ ID NO:6), and wherein said nucleic acid molecule does not specifically hybridize to nucleotides 511-985 of SEQ ID NO:1 or nucleotides 533-1007 of SEQ ID NO:6.

Claim 7 depends from claim 6 and recites: A method of detecting PCA3 nucleic acid in a sample comprising: a) contacting said sample with the nucleic acid molecule according to claim 6 under conditions such that hybridization occurs; and b) detecting the presence of said molecule bound to PCA3 nucleic acid.

The Approved Product captures PCA3 target RNA molecules in urine specimens by hybridizing capture oligonucleotides to the target RNA. The capture oligonucleotides are complementary to sequence specific regions of the target RNA. The hybridized target RNA is captured onto magnetic particles that are then separated from the urine specimen in a magnetic field. The captured target RNA is washed and amplified using TMA. The TMA reaction is performed using RT and T7 RNA polymerase and a set of primers specific for PCA3. RT produces a DNA copy of the target PCA3 RNA that contains an added T7 promoter. T7 RNA polymerase produces multiple copies of PCA3 RNA amplicon from the DNA copy. The amplicon is detected using HPA and a labeled, single-stranded PCA3 nucleic acid probe that is complementary to the PCA3 amplicon and that hybridizes specifically to it. During HPA, the labeled PCA3 probe is hybridized to the PCA3 amplicon, a selection reagent inactivates the label on unhybridized probe, and the label on the hybridized probe is measured. Thus, in using the Approved Product according to the approved use, a laboratory: a) contacts a sample with the nucleic acid

molecule according to claim 6 under conditions such that hybridization occurs; and b) indirectly detects the presence of the nucleic acid molecule bound to PCA3 nucleic acid.

10. A statement beginning on a new page of the relevant dates and information pursuant to 35 U.S.C. 156(g) in order to enable the Secretary of Health and Human Services or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period as follows:

(a) For a patent claiming a medical device:

(i) The effective date of the investigational device exception (IDE) and the IDE number, if applicable, or the date on which the applicant began first clinical investigation involving the device, if no IDE was submitted, and any available substantiation of that date;

An IDE was not required for the Approved Product. The Institutional Review Board ("IRB") approved the protocol for clinical evaluation of the Approved Product on July 24, 2009. A copy of the IRB protocol approval letter is attached as **Exhibit 15**. Thus, the "regulatory review period" under 35 U.S.C. § 156(g)(3) began on **July 24, 2009**, the date of IRB approval. *See* 21 C.F.R. § 60.22(c).

(ii) The date on which the application for product approval or notice of completion of a product development protocol under Section 515 of the Federal Food, Drug and Cosmetic Act was initially submitted and the number of the application; and

The Application for Premarket Approval ("PMA") for the Approved Product was initially submitted to the FDA by Gen-Probe on August 10, 2010 and given PMA number P100033. *See* FDA letter dated September 8, 2010 **(Exhibit 16)**. This letter establishes **August 10, 2010**, as the initial submission date of the PMA for the Approved Product for purposes of 35 U.S.C. § 156(g)(3).

(iii) The date on which the application was approved or the protocol declared to be completed;

The PMA for the Approved Product was approved by the FDA on February 13, 2012. *See* FDA letter dated February 13, 2012 **(Exhibit 14)**. This establishes the end of the "regulatory review period" under 35 U.S.C. 156(g)(3) as **February 13, 2012**.

11. A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities;

A listing of the significant activities undertaken by the Marketing Applicant, and their respective dates, with respect to the Approved Product during the applicable regulatory review period is attached as **Exhibits 17 and 18**, the disclosure of which is incorporated herein in its entirety.

12. A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as to the length of extension claimed, including how the length of extension was determined;

Statement That the Patent Is Eligible For Extension

In the opinion of the Applicants, the '765 patent is eligible for extension under 35 U.S.C. § 156(a) because it satisfies all of the requirements for such extension as follows:

(1) 35 U.S.C. 156(a)

The '765 patent claims the Approved Product and its use as detailed in Section (9) above.

(2) 35 U.S.C. 156 (a)(l)

The '765 patent was granted on March 7, 2006 on an earliest filed international application filed on April 9, 1998. As such, the '765 patent expires on April 9, 2018, which is 20 years from the earliest non-provisional United States filing date. This application has therefore been submitted before the expiration of the term of the '765 patent.

(3) 35 U.S.C. 156(a)(2)

The term of the '765 patent has never been extended.

(4) 35 U.S.C. 156(a)(3)

This application is being submitted by Gen-Probe, as agent for the Applicants (see **Exhibits 10-11**), the owners of record of the '765 patent through assignments from the inventors as detailed on pages 1-2 above and in **Exhibits 4-8**, in accordance with the requirements of 35 U.S.C. § 156(d) and rules of the U.S. Patent and Trademark Office.

(5) 35 U.S.C. 156(a)(4)

As evidenced by the February 13, 2012 approval letter from the FDA (**Exhibit 14**), the Approved Product was subject to a regulatory review period under Section 515 of the FFDCA before its commercial marketing or use.

(6) **35 U.S.C. 156(a)(5)(A)**

The permission for commercial marketing of the Approved Product after this regulatory review period is the first permitted commercial marketing of the Approved Product, under provision of the FFDCA (21 U.S.C. § 360e) under which the regulatory review period occurred, as confirmed by the absence of any approved PMA for the Approved Product prior to February 13, 2012.

(7) **35 U.S.C. 156(c)(4)**

No other patent has been extended for the same regulatory review period for the Approved Product.

Statement Regarding Length of Extension Claimed

The term of the '765 patent should be extended **745 days** from April 9, 2018 to **April 23, 2020**. In accordance with the implementing regulations of 37 C.F.R. 1.777 with respect to patent term extensions for a medical device, the term extension of the '765 patent based on the regulatory review of PROGENSA was determined as follows:

> **Section 1.777 Calculation of patent term extension for a medical device.**
>
> **(a) If a determination is made pursuant to § 1.750 that a patent for a medical device is eligible for extension, the term shall be extended by the time as calculated in days in the manner indicated by this section. The patent term extension will run from the original expiration date of the patent or earlier date as set by terminal disclaimer (§1.321).**
>
> The original expiration date of the '765 patent is April 9, 2018.
>
> **(b) The term of the patent for a medical device will be extended by the length of the regulatory review period for the product as determined by the Secretary of Health and Human Services, reduced as appropriate pursuant to paragraphs (d)(1) through (d)(6) of this section.**
>
> **(c) The length of the regulatory review period for a medical device will be determined by the Secretary of Health and Human Services. Under 35 U.S.C. 156(g)(3)(B), it is the sum of —**

(1) The number of days in the period beginning on the date a clinical investigation on humans involving the device was begun and ending on the date an application was initially submitted with respect to the device under section 515 of the Federal Food, Drug, and Cosmetic Act; and

(2) The number of days in the period beginning on the date the application was initially submitted with respect to the device under section 515 of the Federal Food, Drug, and Cosmetic Act, and ending on the date such application was approved under such Act or the period beginning on the date a notice of completion of a product development protocol was initially submitted under section 515(f)(5) of the Act and ending on the date the protocol was declared completed under section 515(f)(6) of the Act.

The number of days in the testing period of paragraph (c)(1) extends from the IRB approval date on July 24, 2009, to the filing date of PMA P100033 on August 10, 2010, being **383 days**.

The number of days in the PMA approval period of paragraph (c)(2) extends from the filing of PMA P100033 on August 10, 2010, to the date of approval of PMA P100033 on February 13, 2012, being **553 days**.

The regulatory review period is the sum of the periods of paragraphs (c)(1) and (c)(2), being **936 days**.

(d) The term of the patent as extended for a medical device will be determined by —

(1) Subtracting from the number of days determined by the Secretary of Health and Human Services to be in the regulatory review period pursuant to paragraph (c) of this section:

(i) The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section which were on and before the date on which the patent issued;

(ii) The number of days in the periods of paragraphs (c)(1) and (c)(2) of this section during which it is determined under 35 U.S.C. 156(d)(2)(B) by the Secretary of Health and Human Services that applicant did not act with due diligence;

(iii) One-half the number of days remaining in the period defined by paragraph (c)(1) of this section after that period is reduced in accordance with paragraphs (d)(1) (i) and (ii) of this section; half days will be ignored for purposes of subtraction;

With respect to paragraph (d)(1)(i), the number of days in the periods of paragraphs (c)(1) and (c)(2) on and before March 7, 2006, the day on which the '765 patent issued, is **0 (zero) days**.

With respect to paragraph (d)(1)(ii), there has been no such determination by the Secretary, and thus the number of days under (d)(1)(ii) is **0 (zero) days**.

With respect to paragraph (d)(1)(iii), one-half the number of days remaining in the period defined by paragraph (c)(1) – **383 days** – after that period is reduced in accordance with paragraphs (d)(1)(i) – **0 (zero) days** – and (d)(1)(ii) – **0 (zero) days** – is **191 days**, ignoring the half day.

Subtracting from the regulatory review period of **936 days** as determined above pursuant to section 1.777(c) the number of days determined above with respect to sections 1.777(d)(1)(i), (ii) and (iii), the term of patent extension for the '765 patent is **936 days** minus **0 (zero) days** minus **0 (zero) days** minus **191 days** for a total of **745 days**.

(2) By adding the number of days determined in paragraph (d)(1) of this section to the original term of the patent as shortened by any terminal disclaimer;

The original expiration date of the '765 patent is April 9, 2018. Adding the **745 days** determined in sections 1.777(d)(1) to the original term of the patent results in an extended term to **April 23, 2020**.

(3) By adding 14 years to the date of approval of the application under section 515 of the Federal Food, Drug, and Cosmetic Act or the

date a product development protocol was declared completed under section 515(f)(6) of the Act;

Adding 14 years to the February 13, 2012, date of approval of the PMA results in the date February 13, 2026.

(4) By comparing the dates for the ends of the periods obtained pursuant to paragraphs (d)(2) and (d)(3) of this section with each other and selecting the earlier date;

The earlier date of **April 23, 2020**, and February 13, 2026, is **April 23, 2020**.

(5) If the original patent was issued after September 24, 1984,

(i) By adding 5 years to the original expiration date of the patent or earlier date set by terminal disclaimer; and

(ii) By comparing the dates obtained pursuant to paragraphs (d)(4) and (d)(5)(i) of this section with each other and selecting the earlier date;

Adding 5 years to the original expiration date of the patent of April 9, 2018, gives a date of April 9, 2023. The earlier date of **April 23, 2020**, and April 9, 2023, is **April 23, 2020**.

Thus, as calculated above, the term of the '765 patent is eligible for a **745 day** extension to **April 23, 2020**.

13. **A statement that applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought (see §1.765);**

Applicants acknowledge a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought.

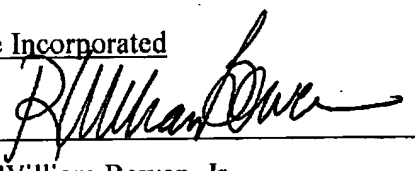
14. **The prescribed fee for receiving and acting upon the application for extension (see §1.20(j)); and**

The Patent and Trademark Office is authorized to charge the filing fee of $1,120.00 and any additional fees which may be required by this or any other related paper, or to credit any overpayment to Deposit Account No. 19-0036.

15. **The name, address, and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed.**

Eldora L. Ellison, Reg. No. 39,967
Helene C. Carlson, Reg. No. 47,473
Sterne, Kessler, Goldstein & Fox, PLLC
1100 New York Avenue, NW
Washington, DC 20005
Tel (202) 371-2600 Fax (202) 371-2540

FOR: Gen-Probe Incorporated

SIGNATURE: 

BY: R. William Bowen, Jr.

TITLE: Sr. Vice President and General Counsel

DATE: Apr. 5, 2011

1491392.2

US007008765B1

Exhibit 1

(12) **United States Patent**
Bussemakers et al.

(10) **Patent No.: US 7,008,765 B1**
(45) **Date of Patent: Mar. 7, 2006**

(54) **PCA3, PCA3 GENES, AND METHODS OF USE**

(75) Inventors: **Marion J. G. Bussemakers**, Nijmegen (NL); **William B. Isaacs**, Glyndon, MD (US)

(73) Assignees: **The Johns Hopkins University**, Baltimore, MD (US); **Stichting Katholieke Universiteit more particularly The University Medical Centre**, Nijmegen (NL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/402,713**

(22) PCT Filed: **Apr. 9, 1998**

(86) PCT No.: **PCT/CA98/00346**

§ 371 (c)(1),
(2), (4) Date: **Jun. 13, 2000**

(87) PCT Pub. No.: **WO98/45420**

PCT Pub. Date: **Oct. 15, 1998**

Related U.S. Application Data

(60) Provisional application No. 60/041,836, filed on Apr. 10, 1997.

(51) **Int. Cl.**

C12Q 1/68	(2006.01)
C07H 21/04	(2006.01)
C12N 15/11	(2006.01)
C12N 15/63	(2006.01)

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(58) **Field of Classification Search** 536/23.1, 536/24.3, 23.5, 24.1, 24.33; 435/320.1
See application file for complete search history.

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(74) *Attorney, Agent, or Firm*—Sterne,Kessler,Goldstein & Fox PLLC

(57) **ABSTRACT**

The present invention relates, in general, to a prostate-specific antigen, PCA3. In particular, the present invention relates to nucleic acid molecules coding for the PCA3 protein; purified PCA3 proteins and polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antibodies having binding affinity specifically to PCA3 proteins and polypeptides; hybridomas containing the antibodies; nucleic acid probes for the detection of nucleic acids encoding PCA3 proteins; a method of detecting nucleic acids encoding PCA3 proteins or polypeptides in a sample; kits containing nucleic acid probes or antibodies; bioassays using the nucleic acid sequence, protein or antibodies of this invention to diagnose, assess, or prognose a mammal afflicted with prostate cancer; therapeutic uses; and methods of preventing prostate cancer in an animal.

39 Claims, 17 Drawing Sheets

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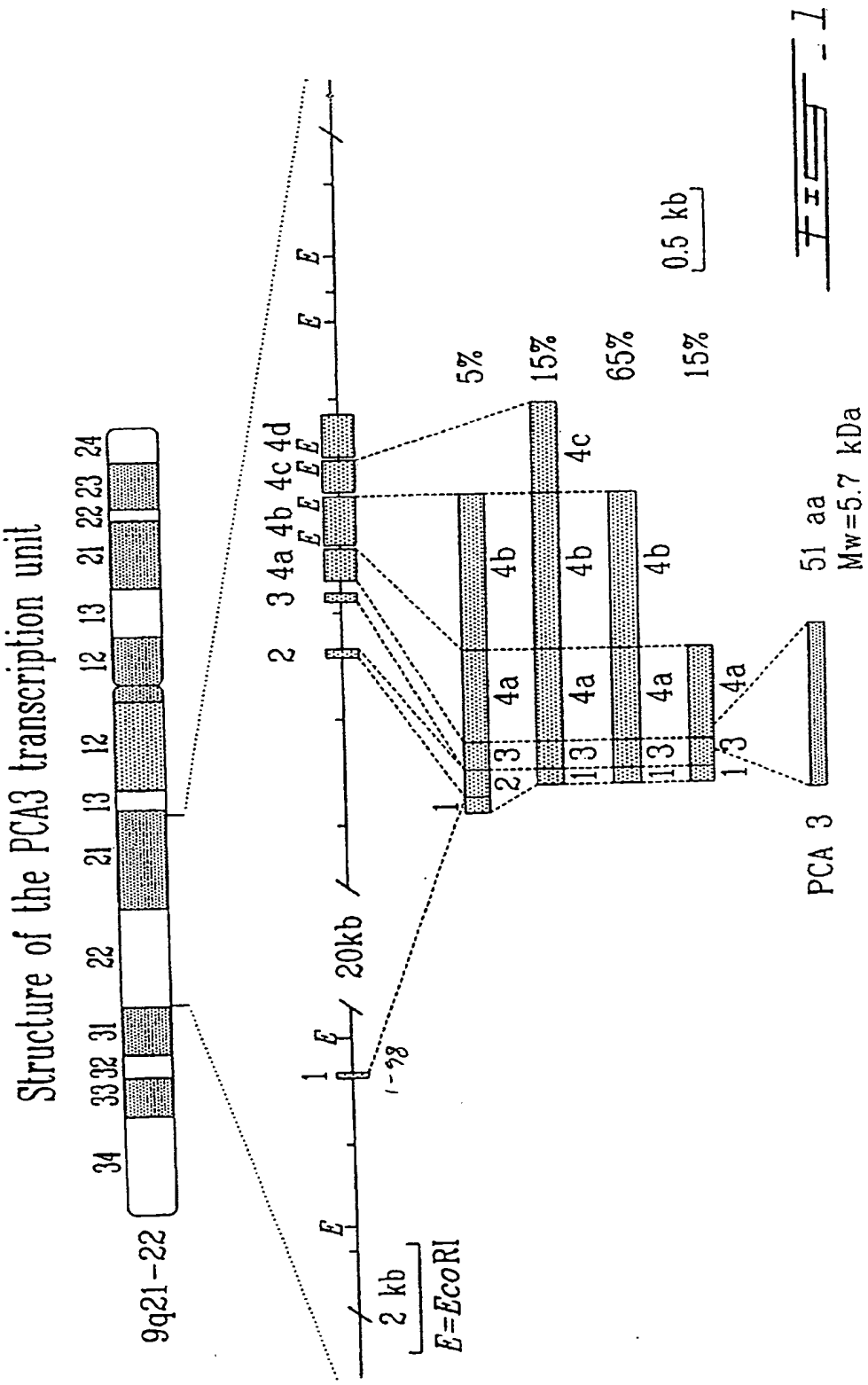
Structure of the PCA3 transcription unit
9q21-22
34
33 32 31
22
21 13
12 12
13 21
22 23 24
E
1
20kb
2 kb
E=EcoRI
2 3 4a 4b 4c 4d
E E E E E
5%
15%
65%
15%
0.5 kb
4c
4b
4a
PCA 3
51 aa
Mw=5.7 kDa
Exon 1

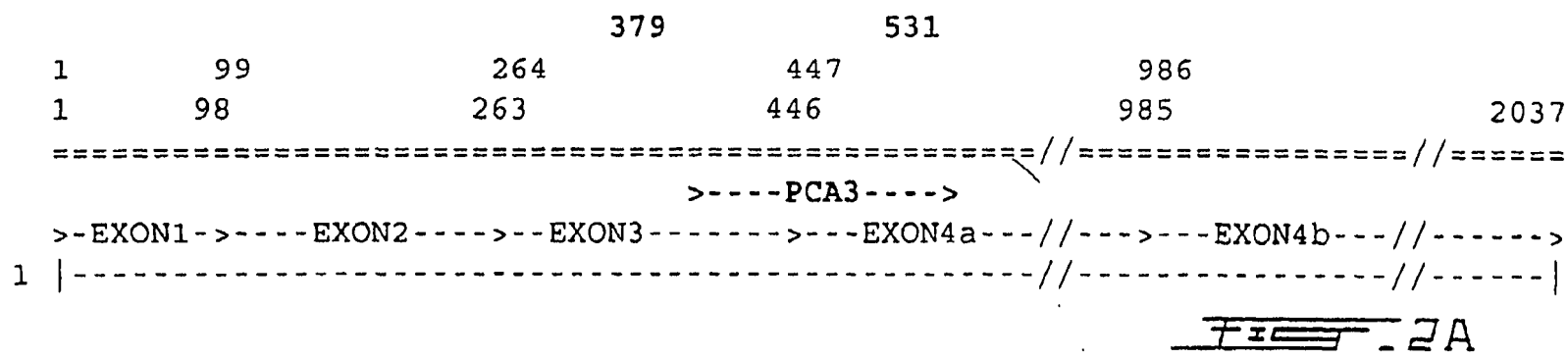

FIG. 2A

1) EXON1, 2) EXON2, 3) EXON3, 4) EXON4A, 5) EXON4b, **PCA3 (amino acids)**
(poly-A-addition signals are underlined)
CON) : Range= 1 to 2037

Name Base

1 1 agaagctggc atcagaaaaa cagaggggag atttgtgtgg ctgcagccga gggagaccag

CON 1 AGAAGCTGGC ATCAGAAAAA CAGAGGGGAG ATTTGTGTGG CTGCAGCCGA GGGAGACCAG

1 61 gaagatctgc atggtgggaa ggacctgatg atacagag

2 1 ga attacaacac atatacttag

FIG. 2B

```
CON 61    GAAGATCTGC ATGGTGGGAA GGACCTGATG ATACAGAGGA ATTACAACAC ATATACTTAG

2   23    tgtttcaatg aacaccaaga taaataagtg aagagctagt ccgctgtgag tctcctcagt

CON 121   TGTTTCAATG AACACCAAGA TAAATAAGTG AAGAGCTAGT CCGCTGTGAG TCTCCTCAGT

2   83    gacacagggc tggatcacca tcgacggcac tttctgagta ctcagtgcag caaagaaag

CON 181   GACACAGGGC TGGATCACCA TCGACGGCAC TTTCTGAGTA CTCAGTGCAG CAAAGAAAG

2   142   actacagaca tctcaatggc aggg
3   1                                 gtgaga aataagaaag gctgctgact ttaccatctg

CON 240   ACTACAGACA TCTCAATGGC AGGGGTGAGA AATAAGAAAG GCTGCTGACT TTACCATCTG

3   37    aggccacaca tctgctgaaa tggagataat taacatcact agaaacagca agatgacaat

CON 300   AGGCCACACA TCTGCTGAAA TGGAGATAAT TAACATCACT AGAAACAGCA AGATGACAAT
```

FIG. 2C

```
3    97    ataatgtcta agtagtgac atgtttttg cacatttcc agccccttt aaatatcca cacaca

CON 360    ATAATGTCTA AGTAGTGAC ATGTTTTTG CACATTTCC AGCCCCTTT AAATATCCA CACACA
PCA3  1                          M  F  L  H  I  S  S  P  F  K  Y  P  H  T

3    158   caggaagca caaaaggaa gcacagag
4    1                                 a tccctggga gaaatgccc ggccgccat cttggg

CON 421    CAGGAAGCA CAAAAGGAA GCACAGAGA TCCCTGGGA GAAATGCCC GGCCGCCAT CTTGGG
PCA3 15     Q  E  A  Q  K  E  A  Q  R  S  L  G  E  M  P  G  R  H  L  G


4    35    tcatcgatg agcctcgcc ctgtgcctg gtcccgctt gtgAGGGAA GGACATTAG AAA

CON 481    TCATCGATG AGCCTCGCC CTGTGCCTG GTCCCGCTT GTGAGGGAA GGACATTAG AAA
PCA3 35     S  S  M  S  L  A  L  C  L  V  P  L  V  R  E  G  H ***

4    93    ATGAATTGAT GTGTTCCTTA AAGGATGGGC AGGAAAACAG ATCCTGTTGT GGATATTTAT

CON 538    ATGAATTGAT GTGTTCCTTA AAGGATGGGC AGGAAAACAG ATCCTGTTGT GGATATTTAT
```

Fig. 2D

```
4   153  TTGAACGGGA TTACAGATTT GAAATGAAGT CACAAAGTGA GCATTACCAA TGAGAGGAAA

CON 598  TTGAACGGGA TTACAGATTT GAAATGAAGT CACAAAGTGA GCATTACCAA TGAGAGGAAA

4   213  ACAGACGAGA AAATCTTGAT GGCTTCACAA GACATGCAAC AAACAAAATG GAATACTGTG

CON 658  ACAGACGAGA AAATCTTGAT GGCTTCACAA GACATGCAAC AAACAAAATG GAATACTGTG

4   273  ATGACATGAG GCAGCCAAGC TGGGGaggag ataaccacgg ggcaGAGGGT CAGGATTCTG

CON 718  ATGACATGAG GCAGCCAAGC TGGGGAGGAG ATAACCACGG GGCAGAGGGT CAGGATTCTG

4   333  GCCCTGCTGC CTAAACTGTG CGTTCATAAC CAAATCATTT CATATTTCTA ACCCTCAAAA

CON 778  GCCCTGCTGC CTAAACTGTG CGTTCATAAC CAAATCATTT CATATTTCTA ACCCTCAAAA
```

FIG - 2E

```
4    393   CAAAGCTGTT GTAATATCTG ATCTCTACGG TTCCTTCTGG GCCCAACATT CTCCATATAT

CON  838   CAAAGCTGTT GTAATATCTG ATCTCTACGG TTCCTTCTGG GCCCAACATT CTCCATATAT


4    453   CCAGCCACAC TCATTTTTAA TATTTAGTTC CCAGATCTGT ACTGTGACCT TTCTACACTG

CON  898   CCAGCCACAC TCATTTTTAA TATTTAGTTC CCAGATCTGT ACTGTGACCT TTCTACACTG


4    513   TAGAATAACA TTACTCATTT TGTTCAAA
5    1                                   GA CCCTTCGTGT TGCTGCCTAA TATGTAGCTG

CON  958   TAGAATAACA TTACTCATTT TGTTCAAAGA CCCTTCGTGT TGCTGCCTAA TATGTAGCTG


5    33    ACTGTTTTTC CTAAGGAGTG TTCTGGCCCA GGGGATCTGT GAACAGGCTG GGAAGCATCT

CON  1018  ACTGTTTTTC CTAAGGAGTG TTCTGGCCCA GGGGATCTGT GAACAGGCTG GGAAGCATCT


5    93    CAAGATCTTT CCAGGGTTAT ACTTACTAGC ACACAGCATG ATCATTACGG AGTGAATTAT
```

Fig. 2F

```
CON 1078  CAAGATCTTT CCAGGGTTAT ACTTACTAGC ACACAGCATG ATCATTACGG AGTGAATTAT

5   153   CTAATCAACA TCATCCTCAG TGTCTTTGCC CATACTGAAA TTCATTTCCC ACTTTTGTGC

CON 1138  CTAATCAACA TCATCCTCAG TGTCTTTGCC CATACTGAAA TTCATTTCCC ACTTTTGTGC

5   213   CCATTCTCAA GACCTCAAAA TGTCATTCCA TTAATATCAC AGGATTAACT TTTTTTTTTA

CON 1198  CCATTCTCAA GACCTCAAAA TGTCATTCCA TTAATATCAC AGGATTAACT TTTTTTTTTA

5   273   ACCTGGAAGA ATTCAATGTT ACATGCAGCT ATGGGAATTT AATTACATAT TTTGTTTTCC

CON 1258  ACCTGGAAGA ATTCAATGTT ACATGCAGCT ATGGGAATTT AATTACATAT TTTGTTTTCC

5   333   AGTGCAAAGA TGACTAAGTC CTTTATCCCT CCCCTTTGTT TGATTTTTTT TCCAGTATAA

CON 1318  AGTGCAAAGA TGACTAAGTC CTTTATCCCT CCCCTTTGTT TGATTTTTTT TCCAGTATAA
```

FIG. 2G

```
5    393  AGTTAAAATG CTTAGCCTTG TACTGAGGCT GTATACAGCA CAGCCTCTCC CCATCCCTCC

CON  1378 AGTTAAAATG CTTAGCCTTG TACTGAGGCT GTATACAGCA CAGCCTCTCC CCATCCCTCC

5    453  AGCCTTATCT GTCATCACCA TCAACCCCTC CCATNYSACC TAAACAAAAT CTAACTTGTA

CON  1438 AGCCTTATCT GTCATCACCA TCAACCCCTC CCATNYSACC TAAACAAAAT CTAACTTGTA

5    513  ATTCCTTGAA CATGTCAGGN CATACATTRT TCCTTCTGCC TGAGAAGCTC TTCCTTGTCT

CON  1498 ATTCCTTGAA CATGTCAGGN CATACATTRT TCCTTCTGCC TGAGAAGCTC TTCCTTGTCT

5    573  CTTAANTCTA GAATGATGTA AAGTTTTGAA TAAGTTGACT ATCTTACTTC ATGCAAAGAA

CON  1558 CTTAANTCTA GAATGATGTA AAGTTTTGAA TAAGTTGACT ATCTTACTTC ATGCAAAGAA
```

Fig - 2H

```
5    633   GGGACACATA TGAGATTCAT CATCACATGA GACAGCAAAT ACTAAAAGTG TAATTTGATT

CON  1618  GGGACACATA TGAGATTCAT CATCACATGA GACAGCAAAT ACTAAAAGTG TAATTTGATT

5    693   ATAAGAGTTT AGATAAATAT ATGAAATGCA AGAKCCACAG AGGGAATGTT TATGGGGCAC

CON  1678  ATAAGAGTTT AGATAAATAT ATGAAATGCA AGAKCCACAG AGGGAATGTT TATGGGGCAC

5    753   GTTTGTAAGC CTGGGATGTG AAGMAAAGGC AGGGAACCTC ATAGTATCTT ATATAATATA

CON  1738  GTTTGTAAGC CTGGGATGTG AAGMAAAGGC AGGGAACCTC ATAGTATCTT ATATAATATA

5    813   CTTCATTTCT CTATCTCTAT CACAATATCC AACAAGCTTT TCACAGAATT CATGCAGTGC

CON  1798  CTTCATTTCT CTATCTCTAT CACAATATCC AACAAGCTTT TCACAGAATT CATGCAGTGC

5    873   AAATCCCCAA AGGTAACCTT TATCCATTTC ATGGTGAGTG CGCTTTAGAA TTTTGGCAAA
```

Fig - 21

```
CON 1858  AAATCCCCAA AGGTAACCTT TATCCATTTC ATGGTGAGTG CGCTTTAGAA TTTTGGCAAA

5   933   TCATACTGGT CACTTATCTC AACTTTGAGA TGTGTTTGTC CTTGTAGTTA ATTGAAAGAA

CON 1918  TCATACTGGT CACTTATCTC AACTTTGAGA TGTGTTTGTC CTTGTAGTTA ATTGAAAGAA

5   993   ATAGGGCACT CTTgtgagcc actttagggt tcactcctgg caataaagaa tttacaaaga

CON 1978  ATAGGGCACT CTTgtgagcc actttagggt tcactcctgg caataaagaa tttacaaaga
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FIG - 2J

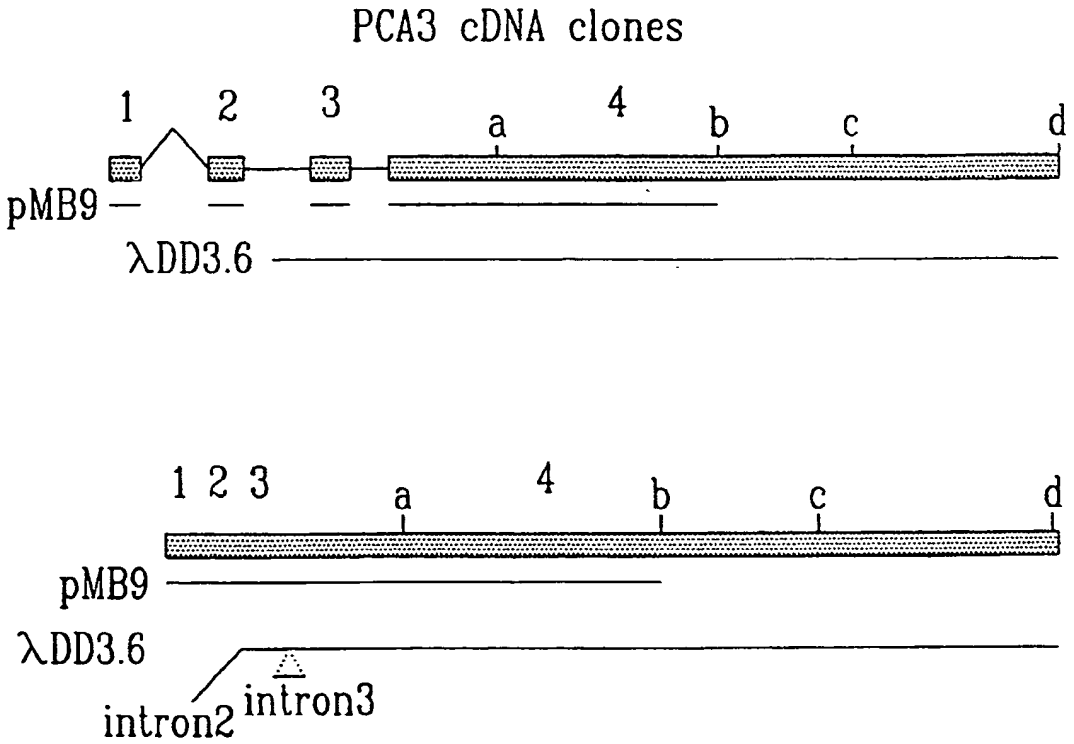
PCA3 cDNA clones
1
2
3
a
4
b
c
d
pMB9
λDD3.6
1 2 3
a
4
b
c
d
pMB9
λDD3.6
intron2
intron3

Fig. 3

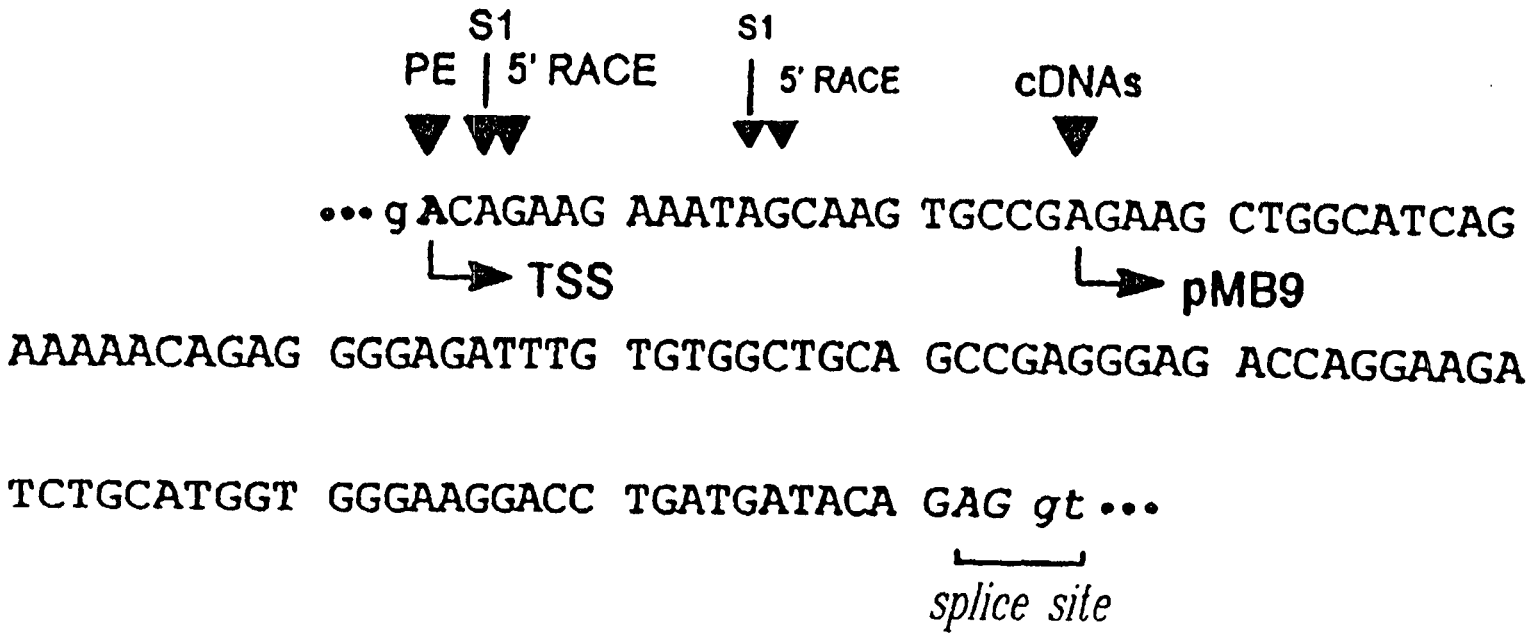
S1
PE
5' RACE
S1
5' RACE
cDNAs
••• g ACAGAAG AAATAGCAAG TGCCGAGAAG CTGGCATCAG
TSS
pMB9
AAAAACAGAG GGGAGATTTG TGTGGCTGCA GCCGAGGGAG ACCAGGAAGA
TCTGCATGGT GGGAAGGACC TGATGATACA GAG gt •••
splice site

Fig. 4

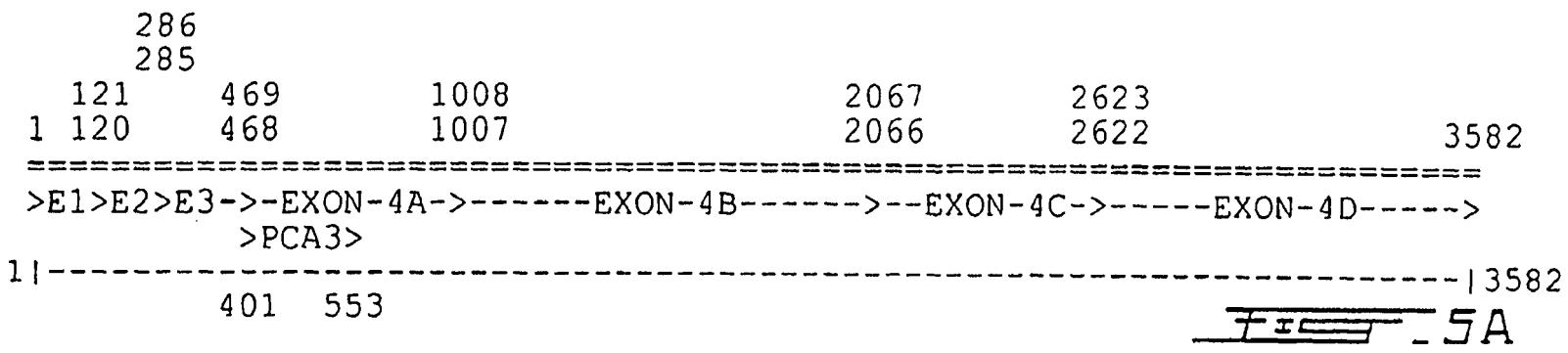

FIG. 5A

Sequence PCA3 cDNA and PCA3 protein:

```
   1    ACAGAAGAAA TAGCAAGTGC CGAGAAGCTG GCATCAGAAA AACAGAGGGG AGATTTGTGT
  61    GGCTGCAGCC GAGGGAGACC AGGAAGATCT GCATGGTGGG AAGGACCTGA TGATACAGAG
 121    GAATTACAAC ACATATACTT AGTGTTTCAA TGAACACCAA GATAAATAAG TGAAGAGCTA
 181    GTCCGCTGTG AGTCTCCTCA GTGACACAGG GCTGGATCAC CATCGACGGC ACTTTCTGAG
 241    TACTCAGTGC AGCAAAGAAA GACTACAGAC ATCTCAATGG CAGGGGTGAG AAATAAGAAA
 301    GGCTGCTGAC TTTACCATCT GAGGCCACAC ATCTGCTGAA ATGGAGATAA TTAACATCAC
```

FIG. 5B

```
  361  TAGAAACAGC AAGATGACAA TATAATGTCT AAGTAGTGAC ATGTTTTTGC ACATTTCCAG
PCA3 1                                        M  F  L     H  I  S  S

  421  CCCCTTTAAA TATCCACACA CACAGGAAGC ACAAAAGGAA GCACAGAGAT CCCTGGGAGA
PCA3 8    P  F  K     Y  P  H     T  Q  E  A     Q  K  E     A  Q  R     S  L  G  E

  481  AATGCCCGGC CGCCATCTTG GGTCATCGAT GAGCCTCGCC CTGTGCCTGG TCCCGCTTGT
PCA3 28   M  P  G     R  H  L     G  S  S  M     S  L  A     L  C  L     V  P  L  V

  541  GAGGGAAGGA CATTAGAAAA TGAATTGATG TGTTCCTTAA AGGATGGGCA GGAAACAGA
PCA3 48   R  E  G     H  *

  601  TCCTGTTGTG GATATTTATT TGAACGGGAT TACAGATTTG AAATGAAGTC ACAAAGTGAG

  661  CATTACCAAT GAGAGGAAAA CAGACGAGAA AATCTTGATG GCTTCACAAG ACATGCAACA

  721  AACAAAATGG AATACTGTGA TGACATGAGG CAGCCAAGCT GGGGAGGAGA TAACCACGGG

  781  GCAGAGGGTC AGGATTCTGG CCCTGCTGCC TAAACTGTGC GTTCATAACC AAATCATTTC

  841  ATATTTCTAA CCCTCAAAAC AAAGCTGTTG TAATATCTGA TCTCTACGGT TCCTTCTGGG

  901  CCCAACATTC TCCATATATC CAGCCACACT CATTTTTAAT ATTTAGTTCC CAGATCTGTA

  961  CTGTGACCTT TCTACACTGT AGAATAACAT TACTCATTTT GTTCAAAGAC CCTTCGTGTT

 1021  GCTGCCTAAT ATGTAGCTGA CTGTTTTTCC TAAGGAGTGT TCTGGCCCAG GGGATCTGTG
```

FIG - 5C

```
1081  AACAGGCTGG GAAGCATCTC AAGATCTTTC CAGGGTTATA CTTACTAGCA CACAGCATGA
1141  TCATTACGGA GTGAATTATC TAATCAACAT CATCCTCAGT GTCTTTGCCC ATACTGAAAT
1201  TCATTTCCCA CTTTTGTGCC CATTCTCAAG ACCTCAAAAT GTCATTCCAT TAATATCACA
1261  GGATTAACTT TTTTTTTTAA CCTGGAAGAA TTCAATGTTA CATGCAGCTA TGGGAATTTA
1321  ATTACATATT TTGTTTTCCA GTGCAAAGAT GACTAAGTCC TTTATCCCTC CCCTTTGTTT
1381  GATTTTTTTT CCAGTATAAA GTTAAAATGC TTAGCCTTGT ACTGAGGCTG TATACAGCAC
1441  AGCCTCTCCC CATCCCTCCA GCCTTATCTG TCATCACCAT CAACCCCTCC CATACCACCT
1501  AAACAAAATC TAACTTGTAA TTCCTTGAAC ATGTCAGGAC ATACATTATT CCTTCTGCCT
1561  GAGAAGCTCT TCCTTGTCTC TTAAATCTAG AATGATGTAA AGTTTTGAAT AAGTTGACTA
1621  TCTTACTTCA TGCAAAGAAG GGACACATAT GAGATTCATC ATCACATGAG ACAGCAAATA
1681  CTAAAAGTGT AATTTGATTA TAAGAGTTTA GATAAATATA TGAAATGCAA GAGCCACAGA
1741  GGGAATGTTT ATGGGGCACG TTTGTAAGCC TGGGATGTGA AGCAAAGGCA GGGAACCTCA
1801  TAGTATCTTA TATAATATAC TTCATTTCTC TATCTCTATC ACAATATCCA ACAAGCTTTT
1861  CACAGAATTC ATGCAGTGCA AATCCCCAAA GGTAACCTTT ATCCATTTCA TGGTGAGTGC
```

Fig. 5D

```
1921  GCTTTAGAAT TTTGGCAAAT CATACTGGTC ACTTATCTCA ACTTTGAGAT GTGTTTGTCC
1981  TTGTAGTTAA TTGAAAGAAA TAGGGCACTC TTGTGAGCCA CTTTAGGGTT CACTCCTGGC
2041  AATAAAGAAT TTACAAAGAG CTACTCAGGA CCAGTTGTTA AGAGCTCTGT GTGTGTGTGT
2101  GTGTGTGTGT GAGTGTACAT GCCAAAGTGT GCCTCTCTCT CTTGACCCAT TATTTCAGAC
2161  TTAAAACAAG CATGTTTTCA AATGGCACTA TGAGCTGCCA ATGATGTATC ACCACCATAT
2221  CTCATTATTC TCCAGTAAAT GTGATAATAA TGTCATCTGT TAACATAAAA AAAGTTTGAC
2281  TTCACAAAAG CAGCTGGAAA TGGACAACCA CAATATGCAT AAATCTAACT CCTACCATCA
2341  GCTACACACT GCTTGACATA TATTGTTAGA AGCACCTCGC ATTTGTGGGT TCTCTTAAGC
2401  AAAATACTTG CATTAGGTCT CAGCTGGGGC TGTGCATCAG GCGGTTTGAG AAATATTCAA
2461  TTCTCAGCAG AAGCCAGAAT TTGAATTCCC TCATCTTTTA GGAATCATTT ACCAGGTTTG
2521  GAGAGGATTC AGACAGCTCA GGTGCTTTCA CTAATGTCTC TGAACTTCTG TCCCTCTTTG
2581  TGTTCATGGA TAGTCCAATA AATAATGTTA TCTTTGAACT GATGCTCATA GGAGAGAATA
2641  TAAGAACTCT GAGTGATATC AACATTAGGG ATTCAAAGAA ATATTAGATT TAAGCTCACA
2701  CTGGTCAAAA GGAACCAAGA TACAAAGAAC TCTGAGCTGT CATCGTCCCC ATCTCTGTGA
```

FIG. 5E

2761 GCCACAACCA ACAGCAGGAC CCAACGCATG TCTGAGATCC TTAAATCAAG GAAACCAGTG

2821 TCATGAGTTG AATTCTCCTA TTATGGATGC TAGCTTCTGG CCATCTCTGG CTCTCCTCTT

2881 GACACATATT AGCTTCTAGC CTTTGCTTCC ACGACTTTTA TCTTTTCTCC AACACATCGC

2941 TTACCAATCC TCTCTCTGCT CTGTTGCTTT GGACTTCCCC ACAAGAATTT CAACGACTCT

3001 CAAGTCTTTT CTTCCATCCC CACCACTAAC CTGAATTGCC TAGACCCTTA TTTTTATTAA

3061 TTTCCAATAG ATGCTGCCTA TGGGCTAATA TTGCTTTAGA TGAACATTAG ATATTTAAAG

3121 TCTAAGAGGT TCAAAATCCA ACTCATTATC TTCTCTTTCT TTCACCTCCC CTGCTCCTCT

3181 CCCTATATTA CTGATTGACT GAACAGGATG GTCCCCAAGA TGCCAGTCAA ATGAGAAACC

3241 CAGTGGCTCC TTGTGGATCA TGCATGCAAG ACTGCTGAAG CCAGAGGATG ACTGATTACG

3301 CCTCATGGGT GGAGGGGACC ACTCCTGGGC CTTCGTGATT GTCAGGAGCA AGACCTGAGA

3361 TGCTCCCTGC CTTCAGTGTC CTCTGCATCT CCCCTTTCTA ATGAAGATCC ATAGAATTTG

3421 CTACATTTGA GAATTCCAAT TAGGAACTCA CATGTTTTAT CTGCCCTATC AATTTTTTAA

3481 ACTTGCTGAA AATTAAGTTT TTTCAAAATC TGTCCTTGTA AATTACTTTT TCTTACAGTG

3541 TCTTGGCATA CTATATCAAC TTTGATTCTT TGTTACAACT TT

Fig. 5F

PCA3, PCA3 GENES, AND METHODS OF USE

This application claims priority to International Application Number PCT/CA98/00346, filed Apr. 9, 1998, and to U.S. Provisional Application No. 60/041,836, filed Apr. 10, 1997. All documents above are herein incorporated by reference in their entirety.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH AND DEVELOPMENT

Statement under MPEP 310. The U.S. government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant No. CA 58236 awarded by the U.S. Department of Health and Human Services National Institutes of Health.

Part of the work performed during development of this invention utilized U.S. Government funds. The U.S. Government has certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates, in general, to a prostate cancer antigen, PCA3. In particular, the present invention relates to nucleic acid molecules coding for the PCA3 protein; purified PCA3 proteins and polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antibodies having binding affinity specifically to PCA3 proteins and polypeptides; hybridomas containing the antibodies; nucleic acid probes for the detection of nucleic acids encoding PCA3 proteins; a method of detecting nucleic acids encoding PCA3 proteins or polypeptides in a sample; kits containing nucleic acid probes or antibodies; bioassays using the nucleic acid sequence, protein or antibodies of this invention to diagnose, assess, or prognose a mammal afflicted with prostate cancer; therapeutic uses; and methods of preventing prostate cancer in an animal.

BACKGROUND OF THE INVENTION

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related deaths in the western male population. When this carcinoma has locally or distantly spread, no curative therapy can be offered. Therefore, efforts to control the disease (i.e., to decrease prostate cancer mortality) have focused on increasing detection of the cancer while it is still locally confined and potentially curable. Studies aimed at the early detection of prostate cancer have demonstrated an appreciable increase in the detection of organ-confined potentially curable prostate cancers. However, it has not yet been demonstrated that the increased detection rate will decrease the prostate cancer-specific mortality rates. On the other hand, there is also no evidence that early diagnosis will decrease the mortality rates. Both in the United States and in Europe, discussions on the efficacy and acceptability of screening programs, the issue of overdiagnosis and overtreatment and the chances that early treatment will lead to reduced prostate cancer morbidity and mortality, are still ongoing and make early detection of prostate cancer a controversial issue (Schröder, *Urology* 46: 6270 (1995)).

Measurements of serum concentrations of prostatic marker enzymes have recognized value in the clinical detection, diagnosis and management of prostate cancer. The two most widely used prostatic marker enzymes are prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA). Normally, both enzymes are secreted from the prostatic epithelial cells into the seminal fluid, but in patients with prostatic disease they leak into the circulation, where they can be detected by means of immunological assays (Armbruster, *Clin. Che.* 39: 181–95 (1993)).

Prostatic acid phosphatase, one of the earliest serum markers for prostate, has an as yet undetermined function and is one of the most predominant protein components in human prostatic secretions. The use of PAP as a marker for prostatic tumors is complicated by the reported structural similarities between the prostate-specific acid phosphatase and the lysosomal acid phosphatase occurring in all tissues. Furthermore, there is a tendency towards lower PAP mRNA and protein levels in prostate cancer in comparison with benign prostatic hyperplasia (BPH). In recent years, PAP measurements were superseded by serum PSA measurements in the clinical management of prostate cancer.

Prostate-specific antigen was identified by several groups in the 1970's as a prostate-specific protein from the seminal fluid. In 1979, it was purified as an antigen from prostate cancer tissue. Further research showed that PSA is produced exclusively by the columnar epithelial cells of the prostate and periuretural glands. Normal prostate epithelium and benign hyperplastic tissue actually produce more PSA mRNA and protein than does prostate cancer tissue. Furthermore, it was shown that loss of differentiation of prostatic carcinomas is associated with a decrease in the level of intraprostatic PSA.

Abnormalities in prostate architecture occurring as a result of prostatic disease lead to an increased leakage of PSA (and PAP) into the serum and make serum PSA measurements a marker for prostate cancer. Despite the fact that early studies have indicated that diagnostic PSA testing would be hampered by the fact that it lacked specificity in differentiating between BPH and prostate cancer, PSA testing was introduced in 1986 and revolutionized the management of patients with prostate cancer. Increased knowledge on the organ specificity of PSA and the relationship of elevated serum PSA levels to prostate disease as well as improvement of biopsy techniques and histological evaluation, led to a appreciation of the clinical value of PSA testing, a utility not yet achieved by any other (prostate) tumor marker. Cloning of the gene that encodes PSA revealed that it is a member of the human kallikrein gene family and resulted in the development of new approaches to the use of PSA as a marker: the very sensitive reverse transcriptaske polymerase chain reaction (RT-PCR) method is used to detect extremely small numbers of malignant prostate cells in blood samples from prostate cancer patients and might provide a sensitive tool to identify patients with micrometastatic disease (Moreno et al., *Cancer Res.* 52: 6110–12 (1992); and Katz et al., *Urology* 43: 765–75 (1994)).

Prostate-specific membrane antigen (PSM) was originally identified using an antibody developed by immunizing mice with the membrane fraction of LNCaP human prostatic adenocarcinoma cells. Like PAP and PSA, PSM can be detected in normal prostate, BPH and prostate cancer and is absent from most other tissues. Also for PSM, RT-PCR studies have been developed to detect circulating prostate cancer cells, however, further investigations are required to establish the usefulness of PSM as marker for prostatic cancer.

In summary, PSA is currently recognized as the premier marker for prostatic cancer, being useful for screening selected populations of patients with symptoms indicative of prostate cancer and for monitoring patients after therapy, especially after surgical prostatectomy (measurable levels of PSA indicate residual disease or metastasis and increasing PSA concentrations indicate recurrent disease). The significant weaknesses of PSA as a tumor marker are that (1) PSA is not able to always distinguish prostate cancer from BPH; and (2) that its expression decreases with loss of differentiation of carcinomas.

In view of the fact that advanced prostate cancer remains a life threatening disease reaching a very significant proportion of the male population, there remains a need for the development of new treatment and diagnostic modalities for (late stage) prostate cancer.

The present invention seeks to meet these and other needs.

The present description refers to a number of documents, the content of which is herein incorporated by reference.

SUMMARY OF THE INVENTION

The invention provides, in general, isolated nucleic acid molecules coding for PCA3 or fragments thereof.

The invention further provides purified polypeptides encoding PCA3 or an epitope binding portion thereof.

The invention also provides nucleic acids for the specific detection of the presence of nucleic acids encoding PCA3 proteins or polypeptides in a sample.

The invention further provides a method of detecting nucleic acid encoding PCA3 in a sample.

The invention also provides a kit for detecting the presence of nucleic acid encoding PCA3 in a sample.

The invention further provides a recombinant nucleic acid molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described isolated nucleic acid molecule.

The invention also provides a recombinant nucleic acid molecule comprising a vector and the above-described isolated nucleic acid molecule.

The invention further provides an antisense PCA3 nucleic acid molecule.

The invention also provides a cell that contains the above-described recombinant nucleic acid molecule.

The invention further provides a non-human organism that contains the above-described recombinant nucleic acid molecule.

The invention also provides an antibody having binding affinity specifically to PCA3 or an epitope-bearing portion thereof.

The invention further provides a method of detecting PCA3 in a sample.

The invention also provides a method of measuring the amount of PCA3 in a sample.

The invention in addition provides immunogenic reagents to induce protection against PCA3-expressing prostate cancer cells. Preferably, such immunogenic reagents are polypeptides encoding PCA3, an antigenic portion thereof, fusion proteins encoding PCA3 or fusion protein encoding antigenic portions of PCA3. In such an embodiment, these immunogenic reagents would function as vaccine agents.

The invention further provides a method of detecting antibodies having binding affinity specifically to PCA3.

The invention further provides a diagnostic kit comprising a first container means containing the above-described antibody, and a second container means containing a conjugate comprising a binding partner of the monoclonal antibody and a label.

The invention also provides a hybridoma which produces the above-described monoclonal antibody.

The invention further provides diagnostic methods for human disease, in particular, prostate cancer. Preferably, a method of diagnosing the presence or predisposition to develop prostate cancer in a patient is provided herein.

The invention also provides methods for therapeutic uses involving all or part of (1) a nucleic acid sequence encoding PCA3, (2) antisense PCA3 nucleic acid molecules, (2) PCA3 protein, or (4) PCA3 antibodies.

Further objects and advantages of the present invention will be clear from the description that follows.

Definitions

In the description that follows, a number of terms used in recombinant DNA (rDNA) technology are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Isolated Nucleic Acid Molecule. An "isolated nucleic acid molecule", as is generally understood and used herein, refers to a polymer of nucleotides, and includes but should not be limited to DNA and RNA. The "isolated" nucleic acid molecule is purified from its natural in vivo state.

Recombinant DNA. Any DNA molecule formed by joining DNA segments from different sources and produced using recombinant DNA technology (aka. molecular genetic engineering).

DNA Segment. A DNA segment, as is generally understood and used herein, refers to a molecule comprising a linear stretch of nucleotides wherein the nucleotides are present in a sequence that can encode, through the genetic code, a molecule comprising a linear sequence of amino acid residues that is referred to as a protein, a protein fragment or a polypeptide.

Gene. A DNA sequence related to a single polypeptide chain or protein, and as used herein includes the 5' and 3' untranslated ends. The polypeptide can be encoded by a full-length sequence or any portion of the coding sequence, so long as the functional activity of the protein is retained.

Complementary DNA (cDNA). Recombinant nucleic acid molecules synthesized by reverse transcription of messenger RNA ("mRNA").

Structural Gene. A DNA sequence that is transcribed into mRNA that is then translated into a sequence of amino acids characteristic of a specific polypeptide.

Restriction Endonuclease. A restriction endonuclease (also restriction enzyme) is an enzyme that has the capacity to recognize a specific base sequence (usually 4, 5, or 6 base pairs in length) in a DNA molecule, and to cleave the DNA molecule at every place where this sequence appears. For example, EcoRI recognizes the double-stranded palindromic base sequence 5'-GAATTC-3'/3'-CTTAAG-5'.

Restriction Fragment. The DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments. Any given genome can be digested by a particular restriction endonuclease into a discrete set of restriction fragments.

Agarose Gel Electrophoresis. To detect a polymorphism in the length of restriction fragments, an analytical method for fractionating double-stranded DNA molecules on the basis of size is required. The most commonly used technique (though not the only one) for achieving such a fractionation is agarose gel electrophoresis. The principle of this method

is that DNA molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules to the greatest extent and the movement of the smallest molecules to the least extent. Note that the smaller the DNA fragment, the greater the mobility under electrophoresis in the agarose gel.

The DNA fragments fractionated by agarose gel electrophoresis can be visualized directly by a staining procedure if the number of fragments included in the pattern is small. The DNA fragments of genomes can be visualized successfully. However, most genomes, including the human genome, contain far too many DNA sequences to produce a simple pattern of restriction fragments. For example, the human genome is digested into approximately 1,000,000 different DNA fragments by EcoRI. In order to visualize a small subset of these fragments, a methodology referred to as the Southern hybridization procedure can be applied.

Southern Transfer Procedure. The purpose of the Southern transfer procedure (also referred to as blotting) is to physically transfer DNA fractionated by agarose gel electrophoresis onto a nitrocellulose filter paper or another appropriate surface or method, while retaining the relative positions of DNA fragments resulting from the fractionation procedure. The methodology used to accomplish the transfer from agarose gel to nitrocellulose involves drawing the DNA from the gel into the nitrocellulose paper by capillary action.

Nucleic Acid Hybridization. Nucleic acid hybridization depends on the principle that two single-stranded nucleic acid molecules that have complementary base sequences will reform the thermodynamically favored double-stranded structure if they are mixed under the proper conditions. The double-stranded structure will be formed between two complementary single-stranded nucleic acids even if one is immobilized on a nitrocellulose filter. In the Southern hybridization procedure, the latter situation occurs. As noted previously, the DNA of the individual to be tested is digested with a restriction endonuclease, fractionated by agarose gel electrophoresis, converted to the single-stranded form, and transferred to nitrocellulose paper, making it available for reannealing to the hybridization probe. Examples of hybridization conditions can be found in Ausubel, F. M. et al., *Current protocols in Molecular Biology*, John Wily & Sons, Inc., New York, N.Y. (1989). A nitrocellulose filter is incubated overnight at 68° C. with labeled probe in a solution containing 50% formamide, high salt (either 5×SSC[20×: 3M NaCl/0.3M trisodium citrate] or 5× SSPE [20×: 3.6M NaCl/0.2M NaH_2PO/0.02M EDTA, pH 7.7]), 5× Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA. This is followed by several washes in 0.2×SSC/0.1% SDS at a temperature selected based on the desired stringency: room temperature (low stringency), 42° C. (moderate stringency) or 68° C. (high stringency). The temperature selected is determined based on the melting temperature (Tm) of the DNA hybrid.

Hybridization Probe. To visualize a particular DNA sequence in the Southern hybridization procedure, a labeled DNA molecule or hybridization probe is reacted to the fractionated DNA bound to the nitrocellulose filter. The areas on the filter that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling are visualized. The hybridization probe is generally produced by molecular cloning of a specific DNA sequence.

Oligonucleotide or Oligomer. A molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three. Its exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. An oligonucleotide can be derived synthetically or by cloning.

Sequence Amplification. A method for generating large amounts of a target sequence. In general, one or more amplification primers are annealed to a nucleic acid sequence. Using appropriate enzymes, sequences found adjacent to, or in between the primers are amplified.

Amplification Primer. An oligonucleotide which is capable of annealing adjacent to a target sequence and serving as an initiation point for DNA synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is initiated.

Antisense nucleic acid molecule. An "antisense nucleic acid molecule" refers herein to a molecule capable of forming a stable duplex or triplex with a portion of its targeted nucleic acid sequence (DNA or RNA). The use of antisense nucleic acid molecules and the design and modification of such molecules is well known in the art as described for example in WO 96/32966, WO 96/11266, WO 94/15646, WO 93/08845, and U.S. Pat. No. 5,593,974. Antisense nucleic acid molecules according to the present invention can be derived from the nucleic acid sequences of the present invention and modified in accordance to well known methods. For example, some antisense molecules can be designed to be more resistant to degradation, to increase their affinity to their targeted sequence, to affect their transport to chosen cell types or cell compartments, and/or to enhance their lipid solubility by using nucleotide analogs and/or substituting chosen chemical fragments thereof, as commonly known in the art.

Vector. A plasmid or phage DNA or other DNA sequence into which DNA can be inserted to be cloned. The vector can replicate autonomously in a host cell, and can be further characterized by one or a small number of endonuclease recognition sites at which such DNA sequences can be cut in a determinable fashion and into which DNA can be inserted. The vector can further contain a marker suitable for use in the identification of cells transformed with the vector. Markers, for example, are tetracycline resistance or ampicillin resistance. The words "cloning vehicle" are sometimes used for "vector."

Expression. Expression is the process by which a structural gene produces a polypeptide. It involves transcription of the gene into mRNA, and the translation of such mRNA into polypeptide(s).

Expression Vector. A vector or vehicle similar to a cloning vector but which is capable of expressing a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences.

Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host and can additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites.

Functional Derivative. A "functional derivative" of a sequence, either protein or nucleic acid, is a molecule that possesses a biological activity (either functional or structural) that is substantially similar to a biological activity of the protein or nucleic acid sequence. A functional derivative of a protein can contain post-translational modifications such as covalently linked carbohydrate, depending on the

necessity of such modifications for the performance of a specific function. The term "functional derivative" is intended to include the "fragments," "segments," "variants," "analogs," or "chemical derivatives" of a molecule.

As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties can improve the molecule's solubility, absorption, biological half life, and the like. The moieties can alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, and the like. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980). Procedures for coupling such moieties to a molecule are well known in the art.

Variant. A "variant" of a protein or nucleic acid is meant to refer to a molecule substantially similar in structure and biological activity to either the protein or nucleic acid. Thus, provided that two molecules possess a common activity and can substitute for each other, they are considered variants as that term is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the amino acid or nucleotide sequence is not identical.

Allele. An "allele" is an alternative form of a gene occupying a given locus on the chromosome.

Mutation. A "mutation" is any detectable change in the genetic material which can be transmitted to daughter cells and possibly even to succeeding generations giving rise to mutant cells or mutant individuals. If the descendants of a mutant cell give rise only to somatic cells in multicellular organisms, a mutant spot or area of cells arises. Mutations in the germ line of sexually reproducing organisms can be transmitted by the gametes to the next generation resulting in an individual with the new mutant condition in both its somatic and germ cells. A mutation can be any (or a combination of detectable, unnatural change affecting the chemical or physical constitution, mutability, replication, phenotypic function, or recombination of one or more deoxyribonucleotides; nucleotides can be added, deleted, substituted for, inverted, or transposed to new positions with and without inversion. Mutations can occur spontaneously and can be induced experimentally by application of mutagens. A mutant variation of a nucleic acid molecule results from a mutation. A mutant polypeptide can result from a mutant nucleic acid molecule.

Species. A "species" is a group of actually or potentially interbreeding natural populations. A species variation within a nucleic acid molecule or protein is a change in the nucleic acid or amino acid sequence that occurs among species and can be determined by DNA sequencing of the molecule in question.

Polyacrylamide Gel Electrophoresis (PAGE). The most commonly used technique (though not the only one) for achieving a fractionation of polypeptides on the basis of size is polyacrylamide gel electrophoresis. The principle of this method is that polypeptide molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules to the greatest extent and the movement of the smallest molecules to the least extent. Note that the smaller the polypeptide fragment, the greater the mobility under electrophoresis in the polyacrylamide gel. Both before and during electrophoresis, the polypeptides typically are continuously exposed to the detergent sodium dodecyl sulfate (SDS), under which conditions the polypeptides are denatured. Native gels are run in the absence of SDS. The polypeptides fractionated by polyacrylamide gel electrophoresis can be visualized directly by a staining procedure if the number of polypeptide components is small.

Western Transfer Procedure. The purpose of the Western transfer procedure (also referred to as blotting) is to physically transfer polypeptides fractionated by polyacrylamide gel electrophoresis onto a nitrocellulose filter paper or another appropriate surface or method, while retaining the relative positions of polypeptides resulting from the fractionation procedure. The blot is then probed with an antibody that specifically binds to the polypeptide of interest.

Purified. A "purified" protein or nucleic acid is a protein or nucleic acid that has been separated from a cellular component. "Purified" proteins or nucleic acids have been purified to a level of purity not found in nature.

Substantially Pure. A "substantially pure" protein or nucleic acid is a protein or nucleic acid preparation that is lacking in all other cellular components.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

FIG. 1 shows the genomic structure of the PCA3 gene.

FIGS. 2A–J, (A) a PCA3 cDNA structure; (B)–(J) a PCA3 nucleotide and amino acid cDNA sequence (SEQ ID NOs:1 and 2).

FIG. 3 is a schematic representation comparing cDNA clones pMB9 and λDD3.6.

FIG. 4 shows the location of a transcription start site (TSS) of PCA3. The transcription start site was determined by primer extension (PE), S1-nuclease mapping (S1), and 5' Rapid Amplification of cDNA Ends (RACE) assays. The sequence in FIG. 4 (SEQ ID NO: 9) is set forth between nucleotides 1 and 120 of SEQ ID NO:6 with a "g" nucleotide and a "gt" dinucleotide at the 5' and 3' ends, respectively.

FIGS. 5A–F (A) a PCA3 cDNA structure; (B)–(F) a PCA3 nucleotide and amino acid cDNA sequence (SEQ ID NOs:6 and 7); putative poly-adenylation signals are underlined.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments with reference to the accompanying drawings which are exemplary and should not be interpreted as limiting the scope of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

For purposes of clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following subsections:

I. Isolated Nucleic Acid Molecules Coding for PCA3 Polypeptides.

II. Purified PCA3 Polypeptides.

III. A Nucleic Acid Probe for the Specific Detection of PCA3 Nucleic Acid.

IV. A Method of Detecting the Presence of PCA3 Nucleic Acid in a Sample.

V. A Kit for Detecting the Presence of PCA3 Nucleic Acid in a Sample.

VI. DNA Constructs Comprising a PCA3 Nucleic Acid Molecule and Cells Containing These Constructs.

VII. An Antibody Having Binding Affinity to a PCA3 Polypeptide and a Hybridoma Containing the Antibody.

VIII. A Method of Detecting a PCA3 Polypeptide or Antibody in a Sample.

IX. A Diagnostic Kit Comprising a PCA3 Protein or Antibody.

X. Diagnostic Screening

XI. Therapeutic Treatments

XII. Transgenic PCA3 Non-human Animals

I. Isolated Nucleic Acid Molecules Coding for PCA3 Polypeptides

In one embodiment, the present invention relates to isolated (purified) PCA3 nucleic acid molecules. Preferably, the PCA3 nucleic acid molecule comprises a polynucleotide sequence at least 90% identical (more preferably, 95%, 96%, 97%, 98%, 99% or 100% identical) to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a PCA3 polypeptide comprising the complete amino acid sequence in SEQ ID NO:2 or SEQ ID NO:7;

(b) a nucleotide sequence encoding a PCA3 polypeptide comprising the complete amino acid sequence encoded by the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 682.97;

(c) a nucleotide sequence encoding a PCA3 polypeptide comprising the complete amino acid sequence encoded by the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 100521; and

(d) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), or (c).

pMB9 is a PCA3 cDNA clone which contains exons 1, 2, 3, 4a and 4b of the PCA3 gene. pMB9 was deposited at the Phabagen Collection, University of Utrecht, Padualaan 8, 3584 CH Utrecht (which is a division of the Centraalbureau voor Schimmelcultures, Oosterstratt1, Postbus 273, 3740 AG Baarn) under the regulations of the Budapest Treaty on Apr. 10, 1997 as accession number CBS 682.97.

λDD3.6 is a PCA3 cDNA clone which contains exons 3, 4a, 4b, 4c, and 4d. λDD3.6 was deposited at the Phabagen Collection, University of Utrecht, Padualaan 8, 3584 CH Utrecht (which is a division of the Centraalbureau voor Schimmelcultures, Oosterstratt1, Postbus 273, 3740 AG Baarn) under the regulations of the Budapest Treaty on Mar. 27, 1998 as accession number CBS 100521.

In one preferred embodiment, the isolated nucleic acid molecule comprises a PCA3 nucleotide sequence with greater than 90% identity or similarity to the nucleotide sequence present in SEQ ID NO:1 (preferably greater than 95%, 96%, 97%, 98%, 99% or 100%). In another preferred embodiment, the isolated nucleic acid molecule comprises the PCA3 coding sequence present in SEQ ID NO:1. In another embodiment, the isolated nucleic acid molecule encodes the PCA3 amino acid sequence present in SEQ ID NO:2 or SEQ ID NO:7. In yet another embodiment, the isolated nucleic acid molecule comprises a PCA3 nucleotide sequence with greater than 90% identity or similarity to the nucleotide sequence present in SEQ ID NO:6 (preferably greater than 95%, 96%, 97%, 98%, 99% or 100%). In another preferred embodiment, the isolated nucleic acid molecule comprises the PCA3 coding sequence present in SEQ ID NO:6.

Also included within the scope of this invention are isolated nucleic acids comprising cDNA splice variants of PCA3 or polynucleotide sequences which are at least 90% identical thereto, preferably at least 95% identical thereto. In view of the fact that virtually all combinations of exons are possible, non-limiting examples of such splice variants include isolated PCA3 nucleic acids comprising exons 1, 2, 3, 4a and 4b (SEQ ID NO:1); exons 1, 3, 4a, 4b, and 4c (SEQ ID NO:3 and region 4c which is contiguous to region 4b, see FIG. 1); exons 1, 3, 4a, 4b, 4c, and 4d (SEQ ID NO:3 and region 4c which is contiguous to region 4b and region 4d which is contiguous to region 4c, see FIG. 1); exons 1, 3, 4a, and 4b (SEQ ID NO:3); exons 1, 3, and 4a (SEQ ID NO:4); exons 1, 2, 3, 4a, 4b, 4c, and 4d (SEQ ID NO:6) Preferably, the PCA3 nucleic acid molecule comprises a polynucleotide sequence at least 90% identical (more preferably, 95%, 96%, 97%, 98%, 99% or 100% identical) to one of the above-described splice variants.

Also included within the scope of this invention are the functional equivalents of the herein-described isolated nucleic acid molecules and derivatives thereof. For example, the nucleic acid sequences depicted in SEQ ID NO:1 or SEQ ID NO:6 can be altered by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as depicted in SEQ ID NO:2 and SEQ ID NO:7 can be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of PCA3 nucleic acid depicted in SEQ ID NO:1, 3, 4 or 6, which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence.

In addition, the nucleic acid sequence can comprise a nucleotide sequence which results from the addition, deletion or substitution of at least one nucleotide to the 5'-end and/or the 3'-end of the nucleic acid formula shown in SEQ ID NO:1, 3, 4, or 6 or a derivative thereof. Any nucleotide or polynucleotide can be used in this regard, provided that its addition, deletion or substitution does not substantially alter the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:7 which is encoded by the nucleotide sequence. Moreover, the nucleic acid molecule of the present invention can, as necessary, have restriction endonuclease recognition sites added to its 5'-end and/or 3'-end. All variations of the nucleotide sequence of the PCA3 nucleotide coding sequence and fragments thereof permitted by the genetic code are, therefore, included in this invention.

Further, it is possible to delete codons or to substitute one or more codons by codons other than degenerate codons to produce a structurally modified polypeptide, but one which has substantially the same utility or activity of the polypeptide produced by the unmodified nucleic acid molecule. As recognized in the art, the two polypeptides are functionally equivalent, as are the two nucleic acid molecules which give rise to their production, even though the differences between the nucleic acid molecules are not related to degeneracy of the genetic code.

A. Isolation of Nucleic Acid

In one aspect of the present invention, isolated nucleic acid molecules coding for polypeptides having amino acid sequences corresponding to PCA3 are provided. In particular, the nucleic acid molecule can be isolated from a biological sample containing PCA3 RNA or DNA.

The nucleic acid molecule can be isolated from a biological sample containing PCA3 RNA using the techniques of cDNA cloning and subtractive hybridization. The nucleic acid molecule can also be isolated from a cDNA library using a homologous probe.

The nucleic acid molecule can be isolated from a biological sample containing genomic DNA or from a genomic

library. Suitable biological samples include, but are not limited to, whole organisms, organs, tissues, blood and cells. The method of obtaining the biological sample will vary depending upon the nature of the sample.

One skilled in the art will realize that genomes can be subject to slight allelic variations between individuals. Therefore, the isolated nucleic acid molecule is also intended to include allelic variations, so long as the sequence is a functional derivative of the PCA3 coding sequence. When a PCA3 allele does not encode the identical sequence to that found in SEQ ID NO:1 or 6, it can be isolated and identified as PCA3 using the same techniques used herein, and especially PCR techniques to amplify the appropriate gene with primers based on the sequences disclosed herein.

One skilled in the art will realize that organisms other than humans will also contain PCA3 genes (for example, eukaryotes; more specifically, mammals, birds, fish, and plants; more specifically, gorillas, rhesus monkeys, and chimpanzees). The invention is intended to include, but not be limited to, PCA3 nucleic acid molecules isolated from the above-described organisms.

B. Synthesis of Nucleic Acid

Isolated nucleic acid molecules of the present invention are also meant to include those chemically synthesized. For example, a nucleic acid molecule with the nucleotide sequence which codes for the expression product of a PCA3 gene can be designed and, if necessary, divided into appropriate smaller fragments. Then an oligomer which corresponds to the nucleic acid molecule, or to each of the divided fragments, can be synthesized. Such synthetic oligonucleotides can be prepared, for example, by the triester method of Matteucci et al., *J. Am. Chem. Soc.* 103:3185–3191 (1981) or by using an automated DNA synthesizer.

An oligonucleotide can be derived synthetically or by cloning. If necessary, the 5'-ends of the oligomers can be phosphorylated using T4 polynucleotide kinase. Kinasing of single strands prior to annealing or for labeling can be achieved using an excess of the enzyme. If kinasing is for the labeling of probe, the ATP can contain high specific activity radioisotopes. Then, the DNA oligomer can be subjected to annealing and ligation with T4 ligase or the like.

II. Purified PCA3 Polypeptides

In another embodiment, the present invention relates to a purified polypeptide (preferably, substantially pure) having an amino acid sequence corresponding to PCA3, or a functional derivative thereof. In a preferred embodiment, the polypeptide has the amino acid sequence set forth in SEQ ID NO:2 or 7 or mutant or species variation thereof, or at least 80% identity or at least 90% similarity thereof (preferably, at least 90%, 95%, 96%, 97%, 98%, or 99% identity or at least 95%, 96%, 97%, 98%, or 99% similarity thereof), or at least 6 contiguous amino acids thereof (preferably, at least 10, 15, 20, 25, or 50 contiguous amino acids thereof).

In a preferred embodiment, the invention relates to PCA3 epitopes. The epitope of these polypeptides is an immunogenic or antigenic epitope. An immunogenic epitope is that part of the protein which elicits an antibody response when the whole protein is the immunogen. An antigenic epitope is a fragment of the protein which can elicit an antibody response. Methods of selecting antigenic epitope fragments are well known in the art. See, Sutcliffe et al., *Science* 219:660–666 (1983). Antigenic epitope-bearing peptides and polypeptides of the invention are useful to raise an immune response that specifically recognizes the polypeptides. Antigenic epitope-bearing peptides and polypeptides of the invention comprise at least 7 amino acids (preferably, 9, 10, 12, 15 or 20 amino acids) of the proteins of the invention. An example of a antigenic peptide is HTQEAQKEAQR (SEQ ID NO:5).

Amino acid sequence variants of PCA3 can be prepared by mutations in the DNA. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence shown in SEQ ID NO:2 or 7. Any combination of deletion, insertion, and substitution can also be made to arrive at the final construct, provided that the final construct possesses the desired activity.

While the site for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, random mutagenesis can be conducted at the target codon or region and the expressed PCA3 variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, site-specific mutagenesis.

Preparation of a PCA3 variant in accordance herewith is preferably achieved by site-specific mutagenesis of DNA that encodes an earlier prepared variant or a nonvariant version of the protein. Site-specific mutagenesis allows the production of PCA3 variants through the use of specific oligonucleotide sequences that encode the DNA sequence of the desired mutation. In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by publications such as Adelman et al., *DNA* 2:183 (1983) and Ausubel et al. "Current Protocols in Molecular Biology", J. Wiley & Sons, NY, N.Y., 1996.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably 1 to 10 residues, and typically are contiguous.

Amino acid sequence insertions include amino and/or carboxyl-terminal fusions of from one residue to polypeptides of essentially unrestricted length, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the complete PCA3 sequence) can range generally from about 1 to 10 residues, more preferably 1 to 5.

The third group of variants are those in which at least one amino acid residue in the PCA3 molecule, and preferably, only one, has been removed and a different residue inserted in its place. Such substitutions preferably are made in accordance with the following Table 1 when it is desired to modulate finely the characteristics of PCA3.

TABLE 1

Original Residue	Exemplary Substitutions
Ala	gly; ser
Arg	lys
Asn	gln; his
Asp	glu
Cys	ser
Gln	asn
Glu	asp
Gly	ala; pro
His	asn; gln
Ile	leu; val
Leu	ile; val
Lys	arg; gln; glu
Met	leu; tyr; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr

TABLE 1-continued

Original Residue	Exemplary Substitutions
Tyr	trp; phe
Val	ile; leu

Substantial changes in functional or immunological identity are made by selecting substitutions that are less conservative than those in Table 1, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions that in general are expected are those in which (a) glycine and/or proline is substituted by another amino acid or is deleted or inserted; (b) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (c) a cysteine residue is substituted for (or by) any other residue; (d) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) a residue having an electronegative charge, e.g., glutamyl or aspartyl; or (e) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

Some deletions and insertions, and substitutions are not expected to produce radical changes in the characteristics of PCA3. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. For example, a variant typically is made by site-specific mutagenesis of the native PCA3 encoding-nucleic acid, expression of the variant nucleic acid in recombinant cell culture, and, optionally, purification from the cell culture, for example, by immunoaffinity adsorption on a column (to absorb the variant by binding it to at least one remaining immune epitope). The activity of the cell lysate or purified PCA3 molecule variant is then screened in a suitable screening assay for the desired characteristic. For example, a change in the immunological character of the PCA3 molecule, such as affinity for a given antibody, is measured by a competitive type immunoassay. Changes in immunomodulation activity are measured by the appropriate assay. Modifications of such protein properties as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers are assayed by methods well known to the ordinarily skilled artisan.

A variety of methodologies known in the art can be utilized to obtain the peptide of the present invention. In one embodiment, the peptide is purified from tissues or cells which naturally produce the peptide. Alternatively, the above-described isolated nucleic acid fragments can be used to express the PCA3 protein in any organism. The samples of the present invention include cells, protein extracts or membrane extracts of cells, or biological fluids. The sample will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts used as the sample.

Any organism can be used as a source for the peptide of the invention, as long as the source organism naturally contains such a peptide. As used herein, "source organism" refers to the original organism from which the amino acid sequence of the subunit is derived, regardless of the organism the subunit is expressed in and ultimately isolated from.

One skilled in the art can readily follow known methods for isolating proteins in order to obtain the peptide free of natural contaminants. These include, but are not limited to: immunochromotography, size-exclusion chromatography, HPLC, ion-exchange chromatography, and immuno-affinity chromatography.

In a preferred embodiment, the purification procedures comprise ion-exchange chromatography and size exclusion chromatography. Any one of a large number of ion-exchange resins known in the art can be employed, including for example, monoQ, sepharose Q, macro-prepQ, AG1-X2, or HQ. Examples of suitable size exclusion resins include, but are not limited to, Superdex 200, Superose 12, and Sephycryl 200. Elution can be achieved with aqueous solutions of potassium chloride or sodium chloride at concentrations ranging from 0.01M to 2.0M.

III. A Nucleic Acid for the Specific Detection of PCA3 Nucleic Acid

In another embodiment, the present invention relates to a nucleic acid for the specific detection of the presence of PCA3 nucleic acid in a sample comprising the above-described nucleic acid molecules or at least a fragment thereof which binds under stringent conditions to PCA3 nucleic acid.

In one prefered embodiment, the present invention relates to an isolated nucleic acid consisting of 10 to 1000 nucleotides (prefererably, 10 to 500, 10 to 100, 10 to 50, 10 to 35, 20 to 1000, 20 to 500, 20 to 100, 20 to 50, or 20 to 35) which hybridizes preferentially to RNA or DNA encoding PCA3 or to a PCA3 gene but not to RNA or DNA of which is not related to PCA3, wherein said nucleic acid probe is or is complementary to a nucleotide sequence consisting of at least 10 consecutive nucleotides (preferably, 15, 18, 20, 25, or 30) from the nucleic acid molecule comprising a polynucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the PCA3 polypeptide comprising the complete amino acid sequence in SEQ ID NO:2 or 7;

(b) a nucleotide sequence encoding the PCA3 polypeptide comprising the complete amino acid sequence encoded by the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 682.97, respectively;

(c) a nucleotide sequence encoding the PCA3 polypeptide comprising the complete amino acid sequence encoded by the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 100521, respectively;

(d) a nucleotide sequence encoding the PCA3 gene comprising the nucleotide sequence in SEQ ID NO:1, 3, 4, or 6;

(e) a nucleotide sequence encoding an exon of the PCA3 gene comprising nucleotides 1–98, 99–263, 264–446, 447–985 or 986–2037 as set forth in SEQ ID NO:1;

(f) a nucleotide sequence encoding an exon of the PCA3 gene comprising nucleotides 1–120, 121–285, 286–468, 469–1007, 1008–2066, 2067–2622 or 2623–3582 as set forth in SEQ ID NO:6;

(g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f);

(h) a nucleotide sequence as previously described above.

Preferably, the nucleic acid does not specifically hybridize to nucleotides 511–985 of SEQ ID NO:1, to nucleotides

567–961 of SEQ ID NO:1, to nucleotides 533–1007 of SEQ ID NO:6, or to nucleotides 589–983 of SEQ ID NO:6.

Complementary sequences are also known as antisense nucleic acids when they comprise sequences which are complementary to the coding strand.

Examples of specific nucleic acid probes which can be used in the present invention are set forth in Table 2, below.

TABLE 2

NUCLEIC ACID PROBES

	Size (no. of bases)	Nucleotides
Exon 1	98	1–98 of SEQ ID NO:1
Exon 2	165	99–263 of SEQ ID NO:1
Exon 3	183	264–446 of SEQ ID NO:1
Exon 4a	539	447–985 of SEQ ID NO:1
Exon 4b	1052	986–2037 of SEQ ID NO:1
Probe 1	20	1–20 of SEQ ID NO:1
Probe 2	30	1–30 of SEQ ID NO:1
Probe 3	40	1–40 of SEQ ID NO:1
Probe 4	20	381–400 of SEQ ID NO:1
Probe 5	30	381–410 of SEQ ID NO:1
Probe 6	20	401–420 of SEQ ID NO:1
Probe 7	30	401–430 of SEQ ID NO:1
Probe 8	20	511–530 of SEQ ID NO:1
Probe 9	30	501–530 of SEQ ID NO:1
Probe 10	20	77–98 of SEQ ID NO:1
Probe 11	20	99–118 of SEQ ID NO:1
Probe 12	20	244–263 of SEQ ID NO:1
Probe 13	20	264–283 of SEQ ID NO:1
Probe 14	20	427–446 of SEQ ID NO:1
Probe 15	20	447–466 of SEQ ID NO:1
Exon 1	120	1–120 of SEQ ID NO:6
Exon 2	165	121–285 of SEQ ID NO:6
Exon 3	183	286–468 of SEQ ID NO:6
Exon 4a	539	469–1007 of SEQ ID NO:6
Exon 4b	1059	1008–2066 of SEQ ID NO:6
Exon 4c	556	2067–2622 of SEQ ID NO:6
Exon 4d	960	2623–3582 of SEQ ID NO:6

Of course, as will be understood by the person of ordinary skill, a multitude of additional probes can be designed from the same or other region of SEQ ID NO:1 as well as from SEQ ID NO:6 and other sequences of the present invention.

The nucleic acid probe can be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain another nucleic acid molecule of the present invention. A chromosomal DNA or cDNA library can be prepared from appropriate cells according to recognized methods in the art (cf. *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

In the alternative, chemical synthesis is carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to N-terminal and C-terminal portions of the PCA3 amino acid sequence. Thus, the synthesized nucleic acid probes can be used as primers in a polymerase chain reaction (PCR) carried out in accordance with recognized PCR techniques, essentially according to *PCR Protocols, A Guide to Methods and Applications*, edited by Michael et al., Academic Press, 1990, utilizing the appropriate chromosomal, cDNA or cell line library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes based on the sequence disclosed herein using methods of computer alignment and sequence analysis known in the art (cf. *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

The hybridization probes of the present invention can be labeled by standard labeling techniques such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and the like. After hybridization, the probes can be visualized using known methods.

The nucleic acid probes of the present invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art.

In one embodiment of the above described method, a nucleic acid probe is immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins, such as polyacrylamide and latex beads. Techniques for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The sample used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample which is compatible with the method utilized.

IV. A Method of Detecting the Presence of PCA3 Nucleic Acid in a Sample

In another embodiment, the present invention relates to a method of detecting the presence of PCA3 nucleic acid in a sample comprising a) contacting the sample with the above-described nucleic acid probe, under specific hybridization conditions such that hybridization occurs, and b) detecting the presence of the probe bound to the nucleic acid molecule. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA or DNA samples from human tissue.

V. A Kit for Detecting the Presence of PCA3 Nucleic Acid in a Sample

In another embodiment, the present invention relates to a kit for detecting the presence of PCA3 nucleic acid in a sample comprising at least one container means having disposed therein the above-described nucleic acid probe. In a preferred embodiment, the kit further comprises other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or steptavidin).

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like.

One skilled in the art will readily recognize that the nucleic acid probes described in the present invention can

readily be incorporated into one of the established kit formats which are well known in the art.

VI. DNA Constructs Comprising a PCA3 Nucleic Acid Molecule and Cells Containing These Constructs

In another embodiment, the present invention relates to a recombinant DNA molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described nucleic acid molecules. In another embodiment, the present invention relates to a recombinant DNA molecule comprising a vector and an above-described nucleic acid molecule.

In another embodiment, the present invention relates to a nucleic acid molecule comprising a transcriptional control region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide, and a transcriptional termination region functional in the cell.

Preferably, the above-described molecules are isolated and/or purified DNA molecules.

In another embodiment, the present invention relates to a cell or non-human organism that contains an above-described nucleic acid molecule.

In another embodiment, the peptide is purified from cells which have been altered to express the peptide.

As used herein, a cell is said to be "altered to express a desired peptide" when the cell, through genetic manipulation, is made to produce a protein which it normally does not produce or which the cell normally produces at low levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression can vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the PCA3 coding sequence can be obtained by the above-described methods. This region can be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding a PCA3 gene, the transcriptional termination signals can be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell can be substituted.

Two DNA sequences (such as a promoter region sequence and a PCA3 coding sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of a PCA3 coding sequence, or (3) interfere with the ability of the PCA3 coding sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence.

The present invention encompasses the expression of the PCA3 coding sequence (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, the most efficient and convenient for the production of recombinant proteins and, therefore, are preferred for the expression of the PCA3 coding sequence.

Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains can also be used, including other bacterial strains. In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host can be used. Examples of suitable plasmid vectors include pBR322, pUC18, pUC19, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors include λgt10, λgt11 and the like; and suitable virus vectors include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.

Recognized prokaryotic hosts include bacteria such as *E. coli, Bacillus, Streptomyces, Pseudomonas, Salmonella, Serratia*, and the like. However, under such conditions, the peptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

To express PCA3 in a prokaryotic cell, it is necessary to operably link the PCA3 coding sequence to a functional prokaryotic promoter. Such promoters can be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the int promoter of bacteriophage λ, the bla promoter of the β-lactamase gene sequence of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene sequence of pBR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage λ (P_L and P_R), the trp, recA, lacZ, lacI, and gal promoters of *E. coli*, the α-amylase (Ulmanen et al., *J. Bacteriol.* 162:176–182 (1985)) and the ç-28-specific promoters of *B. subtilis* (Gilman et al., *Gene sequence* 32:11–20 (1984)), the promoters of the bacteriophages of *Bacillus* (Gryczan, In: *The Molecular Biology of the Bacilli*, Academic Press, Inc., NY (1982)), and *Streptomyces* promoters (Ward et al., *Mol. Gen. Genet.* 203:468–478 (1986)). Prokaryotic promoters are reviewed by Glick (*J. Ind. Microbiol.* 1:277–282 (1987)); Cenatiempo (*Biochimie* 68:505–516 (1986)); and Gottesman (*Ann. Rev. Genet.* 18:415–442 (1984)).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold et al. (*Ann. Rev. Microbiol.* 35:365–404 (1981)).

The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene. As used herein, "cell", "cell line", and "cell culture" can be used interchangeably and all such designations include progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny can not be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant progeny have the same functionality as that of the originally transformed cell.

Host cells which can be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the PCA3 peptide of interest. Suitable hosts include eukaryotic cells.

Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, mammalian cells either in vivo, or in tissue culture. Preferred mammalian cells include HeLa cells, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences.

Another preferred host is an insect cell, for example *Drosophila* larvae. Using insect cells as hosts, the *Drosophila* alcohol dehydrogenase promoter can be used, Rubin, *Science* 240:1453–1459 (1988). Alternatively, baculovirus vectors can be engineered to express large amounts of PCA3 in insect cells (Jasny, *Science* 238:1653 (1987); Miller et al., In: *Genetic Engineering* (1986), Setlow, J. K., et al., eds., *Plenum*, Vol. 8, pp. 277–297).

Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed.

Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes. These enzymes are produced in large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals.

Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes peptides bearing leader sequences (i.e., pre-peptides). For a mammalian host, several possible vector systems are available for the expression of PCA3.

A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals can be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, and the like, can be employed. Transcriptional initiation regulatory signals can be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical (such as metabolite) regulation.

As discussed above, expression of PCA3 in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter of the mouse metallothionein I gene sequence (Hamer et al., *J. Mol. Appl. Gen.* 1:273–288 (1982)); the TK promoter of Herpes virus (McKnight, *Cell* 31:355–365 (1982)); the SV40 early promoter (Benoist et al., *Nature* (*London*) 290: 304–310 (1981)); the yeast gal4 gene sequence promoter (Johnston et al., *Proc. Natl. Acad. Sci.* (*USA*) 79:6971–6975 (1982); Silver et al., *Proc. Natl. Acad. Sci.* (*USA*) 81:5951–5955 (1984)) and the CMV immediate-early gene promoter (Thomsen et al., *Proc. Natl. Acad. Sci* (*USA*) 81:659–663 (1984).

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a PCA3 coding sequence does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the PCA3 coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the PCA3 coding sequence).

A PCA3 nucleic acid molecule and an operably linked promoter can be introduced into a recipient prokaryotic or eukaryotic cell either as a non-replicating DNA (or RNA) molecule, which can either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene can occur through the transient expression of the introduced sequence. Alternatively, permanent expression can occur through the integration of the introduced DNA sequence into the host chromosome.

In one embodiment, a vector is employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker can provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements can also be needed for optimal synthesis of single chain binding protein mRNA. These elements can include splice signals, as well as transcription promoters, enhancer signal sequences, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, *Molec. Cell. Biol.* 3:280 (1983).

In a preferred embodiment, the introduced nucleic acid molecule will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors can be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector can be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic vectors include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184, πVX. Such plasmids are, for example, disclosed by Sambrook (cf. *Molecular Cloning: A Laboratory Manual*, second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989). *Bacillus* plasmids include pC194, pC221, pT127, and the like. Such plasmids are disclosed by

Gryczan (In: *The Molecular Biology of the Bacilli*, Academic Press, NY (1982), pp. 307–329). Suitable *Streptomyces* plasmids include pIJ101 (Kendall et al., *J. Bacteriol.* 169:4177–4183 (1987)), and *streptomyces* bacteriophages such as φC31 (Chater et al., In: *Sixth International Symposium on Actinomycetales Biology*, Akademiai Kaido, Budapest, Hungary (1986), pp. 45–54). *Pseudomonas* plasmids are reviewed by John et al. (*Rev. Infect. Dis.* 8:693–704 (1986)), and Izaki (*Jpn. J. Bacteiol.* 33:729–742 (1978)).

Preferred eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known in the art (Botstein et al., *Miami Wntr. Symp.* 19:265–274 (1982); Broach, In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., p. 445–470 (1981); Broach, *Cell* 28:203–204 (1982); Bollon et al., *J. Clin. Hematol. Oncol.* 10:39–48 (1980); Maniatis, In: *Cell Biology: A Comprehensive Treatise*, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563–608 (1980)).

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) can be introduced into an appropriate host cell by any of a variety of suitable means, i.e., transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the production of PCA3. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like).

VII. An Antibody Having Binding Affinity to a PCA3 Polypeptide and a Hybridoma Containing the Antibody

In another embodiment, the present invention relates to an antibody having binding affinity specifically to a PCA3 polypeptide as described above or specifically to a PCA3 polypeptide binding fragment thereof. An antibody binds specifically to a PCA3 polpeptide or binding fragment thereof if it does not bind to non-PCA3 polypeptides. Those which bind selectively to PCA3 would be chosen for use in methods which could include, but should not be limited to, the analysis of altered PCA3 expression in tissue containing PCA3.

The PCA3 proteins of the present invention can be used in a variety of procedures and methods, such as for the generation of antibodies, for use in identifying pharmaceutical compositions, and for studying DNA/protein interaction.

The PCA3 peptide of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide would be generated as described herein and used as an immunogen.

The antibodies of the present invention include monoclonal and polyclonal antibodies, as well as fragments of these antibodies. The invention further includes single chain antibodies. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment; the Fab' fragments, Fab fragments, and Fv fragments.

Of special interest to the present invention are antibodies to PCA3 which are produced in humans, or are "humanized" (i.e. non-immunogenic in a human) by recombinant or other technology. Humanized antibodies can be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (i.e. chimeric antibodies) (Robinson, R. R. et al., International Patent Publication PCT/US86/02269; Akira, K. et al, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison, S. L. et al., European Patent Application 173,494; Neuberger, M. S. et al., PCT Application WO 86/01533; Cabilly, S. et al., European Patent Application 125,023; Better, M. et al., *Science* 240:1041–1043 (1988); Liu, A. Y. et al., *Proc. Natl. Acad. Sci. USA* 84:3439–3443 (1987); Liu, A. Y. et al., *J. Immunol.* 139:3521–3526 (1987); Sun, L. K. et al., *Proc. Natl. Acad. Sci. USA* 84:214–218 (1987); Nishimura, Y. et al., *Canc. Res.* 47:999–1005 (1987); Wood, C. R. et al., *Nature* 314:446–449 (1985)); Shaw et al., *J. Natl. Cancer Inst.* 80:1553–1559 (1988). General reviews of "humanized" chimeric antibodies are provided by Morrison, S. L. (*Science*, 229:1202–1207 (1985)) and by Oi, V. T. et al., *BioTechniques* 4:214 (1986)). Suitable "humanized" antibodies can be alternatively produced by CDR or CEA substitution (Jones, P. T. et al., *Nature* 321:552–525 (1986); Verhoeyan et al., *Science* 239:1534 (1988); Beidler, C. B. et al., *J. Immunol.* 141:4053–4060 (1988)).

In another embodiment, the present invention relates to a hybridoma which produces the above-described monoclonal antibody. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "*Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*," Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth et al., *J. Immunol. Methods* 35:1–21 (1980)).

Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or interperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal which is immunized, the antigenicity of the polypeptide and the site of injection.

The polypeptide can be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or β-galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz et al., *Exp. Cell Res.* 175:109–124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, supra (1984)).

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the

presence of antibodies with the desired specificity using one of the above-described procedures.

In another embodiment of the present invention, the above-described antibodies are detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such as horse radish peroxidase, alkaline phosphatase, and the like) fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, see (Sternberger et al., *J. Histochem. Cytochem.* 18:315 (1970); Bayer et al., *Meth. Enzym.* 62:308 (1979); Engval et al., *Immunol.* 109:129 (1972); Goding, *J. Immunol. Meth.* 13:215 (1976)). The labeled antibodies of the present invention can be used for in vitro, in vivo, and in situ assays to identify cells or tissues which express a specific peptide.

In another embodiment of the present invention the above-described antibodies are immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir et al., "*Handbook of Experimental Immunology*" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby et al., *Meth. Enzym.* 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for in vitro, in vivo, and in situ assays as well as in immunochromatography.

Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby et al., "Application of Synthetic Peptides: Antisense Peptides", In *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY, pp. 289–307 (1992), and Kaspczak et al., *Biochemistry* 28:9230–8 (1989).

Anti-peptide peptides can be generated in one of two fashions. First, the anti-peptide peptides can be generated by replacing the basic amino acid residues found in the PCA3 peptide sequence with acidic residues, while maintaining hydrophobic and uncharged polar groups. For example, lysine, arginine, and/or histidine residues are replaced with aspartic acid or glutamic acid and glutamic acid residues are replaced by lysine, arginine or histidine.

VIII. A Method of Detecting a PCA3 Polypeptide or Antibody in a Sample

In another embodiment, the present invention relates to a method of detecting a PCA3 polypeptide in a sample, comprising: a) contacting the sample with an above-described antibody (or protein), under conditions such that immunocomplexes form, and b) detecting the presence of the antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample. Altered levels of PCA3 in a sample as compared to normal levels can indicate a specific disease (ex. prostate cancer).

In a further embodiment, the present invention relates to a method of detecting a PCA3 antibody in a sample, comprising: a) contacting the sample with an above-described PCA3 protein, under conditions such that immunocomplexes form, and b) detecting the presence of the protein bound to the antibody or antibody bound to the protein. In detail, the methods comprise incubating a test sample with one or more of the proteins of the present invention and assaying whether the antibody binds to the test sample.

Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available immunological assay formats (such as radioimmunoassays, enzyme-linked immunosorbent assays, diffusion based Ouchterlony, or rocket immunofluorescent assays) can readily be adapted to employ the antibodies of the present invention. Examples of such assays can be found in Chard, *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock et al., *Techniques in Immunocytochemistry, Academic Press, Orlando, Fla. Vol.* 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, *Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is capable with the system utilized.

IX. A Diagnostic Kit Comprising PCA3 Protein or Antibody

In another embodiment of the present invention, a kit is provided which contains all the necessary reagents to carry out the previously described methods of detection.

The kit can comprise: i) a first container means containing an above-described antibody, and ii) second container means containing a conjugate comprising a binding partner of the antibody and a label.

The kit can comprise: i) a first container means containing an above-described protein, and preferably, ii) second container means containing a conjugate comprising a binding partner of the protein and a label. More specifically, a diagnostic kit comprises PCA3 protein as described above, to detect antibodies in the serum of potentially infected animals or humans.

In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound antibodies. Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. The compartmentalized kit can be as described above for nucleic acid probe kits.

One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

X. Diagnostic Screening

It is to be understood that although the following discussion is specifically directed to human patients, the teachings are also applicable to any animal that expresses PCA3.

The diagnostic and screening methods of the invention are especially useful for a patient suspected of being at risk for developing a disease associated with an altered expression level of PCA3 based on family history, or a patient in which it is desired to diagnose a PCA3-related disease (ex. prostate cancer).

According to the invention, presymptomatic screening of an individual in need of such screening is now possible using DNA encoding the PCA3 protein or the PCA3 gene of the invention or fragments thereof. The screening method of the invention allows a presymptomatic diagnosis, including prenatal diagnosis, of the presence of a missing or aberrant PCA3 gene in individuals, and thus an opinion concerning the likelihood that such individual would develop or has developed a PCA3-associated disease. This is especially valuable for the identification of carriers of altered or missing PCA3 genes, for example, from individuals with a family history of a PCA3 -associated disease. Early diagnosis is also desired to maximize appropriate timely intervention.

In one preferred embodiment of the method of screening, a tissue sample would be taken from such individual, and screened for (1) the presence of the "normal" PCA3 gene; (2) the presence of PCA3 mRNA and/or (3) the presence of PCA3 protein. The normal human gene can be characterized based upon, for example, detection of restriction digestion patterns in "normal" versus the patients DNA, including RFLP analysis, using DNA probes prepared against the PCA3 sequence (or a functional fragment thereof taught in the invention. Similarly, PCA3 mRNA can be characterized and compared to normal PCA3 mRNA (a) levels and/or (b) size as found in a human population not at risk of developing PCA3-associated disease using similar probes. Lastly, PCA3 protein can be (a) detected and/or (b) quantitated using a biological assay for PCA3 activity or using an immunological assay and PCA3 antibodies. When assaying PCA3 protein, the immunological assay is preferred for its speed. An (1) aberrant PCA3 DNA size pattern, and/or (2) aberrant PCA3 mRNA sizes or levels and/or (3) aberrant PCA3 protein levels would indicate that the patient is at risk for developing a PCA3-associated disease.

More specifically, a method of diagnosing the presence or predisposition to develop prostate cancer in a patient is provided herein.

The screening and diagnostic methods of the invention do not require that the entire PCA3 DNA coding sequence be used for the probe. Rather, it is only necessary to use a fragment or length of nucleic acid that is sufficient to detect the presence of the PCA3 gene in a DNA preparation from a normal or affected individual, the absence of such gene, or an altered physical property of such gene (such as a change in electrophoretic migration pattern). Preferably, any of the probes as described above are used.

Prenatal diagnosis can be performed when desired, using any known method to obtain fetal cells, including amniocentesis, chorionic villous sampling (CVS), and fetoscopy. Prenatal chromosome analysis can be used to determine if the portion of the chromosome possessing the normal PCA3 gene is present in a heterozygous state.

XI. Therapeutic Treatments

A. Therapeutic Nucleic Acids

A therapeutic nucleic acid as a therapeutic agent can have, but is not limited to, at least one of the following therapeutic effects on a target cell: inhibiting transcription of a DNA sequence; inhibiting translation of an RNA sequence; inhibiting reverse transcription of an RNA or DNA sequence; inhibiting a post-translational modification of a protein; inducing transcription of a DNA sequence; inducing translation of an RNA sequence; inducing reverse transcription of an RNA or DNA sequence; inducing a post-translational modification of a protein; transcription of the nucleic acid as an RNA; translation of the nucleic acid as a protein or enzyme; and incorporating the nucleic acid into a chromosome of a target cell for constitutive or transient expression of the therapeutic nucleic acid.

Therapeutic effects of therapeutic nucleic acids can include, but are not limited to: turning off a defective gene or processing the expression thereof, such as antisense RNA or DNA; inhibiting viral replication or synthesis; gene therapy as expressing a heterologous nucleic acid encoding a therapeutic protein or correcting a defective protein; modifying a defective or underexpression of an RNA such as an hnRNA, an mRNA, a tRNA, or an rRNA; encoding a drug or prodrug, or an enzyme that generates a compound as a drug or prodrug in pathological or normal cells expressing the chimeric receptor; and any other known therapeutic effects.

In the method of treating a PCA3-associated disease (preferably, prostate cancer) in a patient in need of such treatment, a PCA3 gene which is not indicative of a disease state can be provided to the cells of such patient in a manner and amount that permits the expression of the PCA3 protein provided by such gene, for a time and in a quantity sufficient to treat such patient. Preferably, gene replacement ("knock out") technology is used that would replace the disease causing PCA3 gene with a PCA3 gene which does not cause disease (specifically, prostate cancer).

Included as well in the invention are pharmaceutical compositions comprising an effective amount of at least one PCA3 antisense oligonucleotide, in combination with a pharmaceutically acceptable carrier. Such antisense oligos include, but are not limited to, at least one nucleotide sequence of 12–500 bases in length which is complementary to PCA3 exons 1, 2, 3, 4a–4d; a DNA sequence of SEQ ID NO:1, 3, 4, or 6; or a DNA sequence encoding at least 4 amino acids of SEQ ID NO:2 or SEQ ID NO:7.

Alternatively, the PCA3 nucleic acid can be combined with a lipophilic carrier such as any one of a number of sterols including cholesterol, cholate and deoxycholic acid. A preferred sterol is cholesterol.

The PCA3 gene therapy nucleic acids and the pharmaceutical compositions of the invention can be administered by any means that achieve their intended purpose. For example, administration can be by parenteral, subcutaneous, intravenous, intramuscular, intra-peritoneal, or transdermal routes. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

Compositions within the scope of this invention include all compositions wherein the PCA3 antisense oligonucleotide is contained in an amount effective to achieve decreased expression of at least one PCA3 gene. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typically, the PCA3 nucleic acid can be administered to mammals, e.g. humans, at a dose of 0.005 to 1 mg/kg/day, or an equivalent amount of the pharmaceutically acceptable salt thereof, per day of the body weight of the mammal being treated.

Suitable formulations for parenteral administration include aqueous solutions of the PCA3 nucleic acid in water-soluble form, for example, water-soluble salts. In

addition, suspensions of the active compounds as appropriate oily injection suspensions can be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension can also contain stabilizers.

Many vector systems are known in the art to provide such delivery to human patients in need of a gene or protein missing from the cell. For example, retrovirus systems can be used, especially modified retrovirus systems and especially herpes simplex virus systems (Gage et al., U.S. Pat. No. 5,082,670). Such methods are provided for, in, for example, the teachings of Breakefield, X. A. et al., *The New Biologist* 3:203–218 (1991); Huang, Q. et al., *Experimental Neurology* 115:303–316 (1992); WO93/03743; WO90/0944; Taylor, WO 92/06693; Mulligan, R. C., *Science* 260:926–932 (1993); and Brown et al., "Retroviral Vectors," in *DNA Cloning: A Practical Approach*, Volume 3, IRL Press, Washington, D.C. (1987). Delivery of a DNA sequence encoding a normally expressed PCA3 protein will effectively replace the PCA3 gene responsible for the disease state (ex. prostate cancer).

The means by which the vector carrying the nucleic acid can be introduced into the cell include but is not limited to, microinjection, electroporation, transduction, or transfection using DEAE-Dextran, lipofection, calcium phosphate or other procedures known to one skilled in the art (*Molecular Cloning, A Laboratory Manual*, Sambrook et al., eds., Cold Spring Harbor Press, Plainview, N.Y. (1989)).

In another embodiment of this invention, a normal PCA3 gene is expressed as a recombinant gene in a cell, so that the cells can be transplanted into a mammal, preferably a human in need of gene therapy. To provide gene therapy to an individual, a genetic sequence which encodes for all or part of the PCA3 gene is inserted into a vector and introduced into a host cell.

Further gene therapy methods which can be used to transfer nucleic acid to a patient are set forth in Chatterjee and Wong, *Current Topics in Microbiol. and Immuno.*, 218: 61–73 (1996); Zhang, *J. Mol. Med.* 74:191–204 (1996); Schmidt-Wolf and Schmidt-Wolf, *J. of Hematotherapy* 4:551–561 (1995); Shaughnessy et al., *Seminars in Oncology* 23 (1): 159–171 (1996); and Dunbar *Annu. Rev. Med.* 47:11–20 (1996).

Specificity for gene expression in prostate cancer cells can be conferred by using appropriate cell-specific regulatory sequences, such as cell-specific enhancers and promoters.

Thus, gene therapy can be used to alleviate PCA3 related pathology by inhibiting the inappropriate expression of a particular form of PCA3. Moreover, gene therapy can be used to alleviate such pathologies by providing the appropriate expression level of a particular form of PCA3. In this case, particular PCA3 nucleic acid sequences can be coded by DNA or RNA constructs which are administered in the form of viruses, as described above.

B. Antagonists and Agonists of PCA3

The ability of antagonists and agonists of PCA3 to interfere or enhance the activity of PCA3 can be evaluated with cells containing PCA3. An assay for PCA3 activity in cells can be used to determine the functionality of the PCA3 protein in the presence of an agent which may act as antagonist or agonist, and thus, agents that interfere or enhance the activity of PCA3 are identified.

The agents screened in the assays can be, but are not limited to, antibodies, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. These agents can be selected and screened 1) at random, 2) by a rational selection or 3) by design using for example, protein or ligand modeling techniques (preferably, computer modeling).

For random screening, agents such as antibodies, peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to or stimulate/block the activity of the PCA3 protein.

Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the PCA3 protein.

In one embodiment, the present invention relates to a method of screening for an antagonist or agonist which stimulates or blocks the activity of PCA3 comprising:

(a) incubating a cell expressing PCA3 with an agent to be tested; and

(b) assaying the cell for the activity of the PCA3 protein by measuring the agents effect on ATP binding of PCA3.

Any cell may be used in the above assay so long as it expresses a functional form of PCA3 and the PCA3 activity can be measured. The preferred expression cells are eukaryotic cells or organisms. Such cells can be modified to contain DNA sequences encoding PCA3 using routine procedures known in the art. Alternatively, one skilled in the art can introduce mRNA encoding the PCA3 protein directly into the cell.

Using PCA3 ligands (ligands including antagonists and agonists as described above) the present invention further provides a method for modulating the activity of the PCA3 protein in a cell. In general, ligands (antagonists and agonists) which have been identified to block or stimulate the activity of PCA3 can be formulated so that the ligand can be contacted with a cell expressing a PCA3 protein in vivo. The contacting of such a cell with such a ligand results in the in vivo modulation of the activity of the PCA3 proteins. So long as a formulation barrier or toxicity barrier does not exist, ligands identified in the assays described above will be effective for in vivo use.

In another embodiment, the present invention relates to a method of administering PCA3 or a PCA3 ligand (including PCA3 antagonists and agonists) to an animal (preferably, a mammal (specifically, a human)) in an amount sufficient to effect an altered level of PCA3 in the animal. The administered PCA3 or PCA3 ligand could specifically effect PCA3 associated functions. Further, since PCA3 is expressed in prostatic cancer cells, administration of PCA3 or PCA3 ligand could be used to alter PCA3 levels in such cells.

One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can readily be determined. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, and other such variables, to be adjusted by the individual physician. Dosage can vary from 0.001 mg/kg to 50 mg/kg of PCA3 or PCA3 ligand, in one or more administrations daily, for one or several days. PCA3 or PCA3 ligand can be administered parenterally by injection or by gradual perfusion over time. It can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and

emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives can also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science,* 16th Ed., Mack Eds. (1980).

In another embodiment, the present invention relates to a pharmaceutical composition comprising PCA3 or PCA3 ligand in an amount sufficient to alter PCA3 associated activity, and a pharmaceutically acceptable diluent, carrier, or excipient. Appropriate concentrations and dosage unit sizes can be readily determined by one skilled in the art as described above (See, for example, *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton Pa. (1980) and WO 91/19008).

C. Immunotherapy

The present invention provides the above-described PCA3 antibodies (preferably, PCA3 murine antibodies and chimeric PCA3 murine-human antibodies, and fragments and regions thereof) which inhibit or neutralize PCA3 biological activity in vivo and are specific for PCA3. These antibodies can be used for therapeutic purposes in subjects having pathologies or conditions associated with the presence of aberrant PCA3 expression. Antibodies, and fragments, regions and derivatives thereof, of the present invention preferably contain at least one region which recognizes an epitope of PCA3 which has inhibiting and/or neutralizing biological activity in vivo.

Treatment comprises parenterally administering a single or multiple doses of the antibody, fragment or derivative. Preferred for human pharmaceutical use are high affinity potent PCA3-inhibiting and/or neutralizing murine and chimeric antibodies, fragments and regions of this invention.

Monoclonal antibodies of the present invention may be administered by any means that enables the active agent to reach the agent's site of action in the body of a mammal. Because proteins are subject to being digested when administered orally, parenteral administration, i.e., intravenous, subcutaneous, intramuscular, would ordinarily be used to optimize absorption.

Monoclonal antibodies of the present invention may be administered either as individual therapeutic agents or in combination with other therapeutic agents. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosage administered will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a daily dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.5 to 50, and preferably 1 to 10 milligrams per kilogram per day given in divided doses 1 to 6 times a day or in sustained release form is effective to obtain desired results.

Dosage forms (composition) suitable for internal administration generally contain from about 1 milligram to about 500 milligrams of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5–95% by weight based on the total weight of the composition.

For parenteral administration, the antibody can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

The murine and chimeric antibodies, fragments and regions of this invention, their fragments, and derivatives can be used therapeutically as immunoconjugates (see for review: Dillman, R. O., *Ann. Int. Med.* 111:592–603 (1989)). They can be coupled to cytotoxic proteins, including, but not limited to Ricin-A, *Pseudomonas* toxin, and Diphtheria toxin. Toxins conjugated to antibodies or other ligands, are known in the art (see, for example, Olsnes, S. et al., *Immunol. Today* 10:291–295 (1989)). Plant and bacterial toxins typically kill cells by disrupting the protein synthetic machinery.

The antibodies of this invention can be conjugated to additional types of therapeutic moieties including, but not limited to, radionuclides, cytotoxic agents and drugs. Examples of radionuclides which can be coupled to antibodies and delivered in vivo to sites of antigen include ^{212}Bi, ^{131}I, ^{186}Re, and ^{90}Y, which list is not intended to be exhaustive. The radionuclides exert their cytotoxic effect by locally irradiating the cells, leading to various intracellular lesions, as is known in the art of radiotherapy.

Cytotoxic drugs which can be conjugated to antibodies and subsequently used for in vivo therapy include, but are not limited to, daunorubicin, doxorubicin, methotrexate, and Mitomycin C. Cytotoxic drugs interfere with critical cellular processes including DNA, RNA, and protein synthesis. For a fuller exposition of these classes of drugs which are known in the art, and their mechanisms of action, see Goodman, A. G., et al., *Goodman and Gilman's THE PHARMACOLOGICAL BASIS OF THERAPEUTICS,* 7th Ed., Macmillan Publishing Co., 1985.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or murine and chimeric antibodies, fragments and regions, or with lymphokines or hemopoietic growth factors, etc., which serve to increase the number or activity of effector cells which interact with the antibodies.

XII. Transgenic PCA3 Non-Human Animals

Methods of Generating Transgenic Non-Human Animals

The non-human animals of the invention comprise any animal having a transgenic interruption or alteration of the endogenous gene(s) (knock-out animals) and/or into the genome of which has been introduced one or more transgenes that direct the expression of human PCA3. Also prefered are the introduction of antisense PCA3 nucleic acids.

Such non-human animals include vertebrates such as rodents, non-human primates, sheep, dog, cow, amphibians, reptiles, etc. Preferred non-human animals are selected from non-human mammalian species of animals, most preferably, animals from the rodent family including rats and mice, most preferably mice.

The transgenic animals of the invention are animals into which has been introduced by nonnatural means (i.e., by human manipulation), one or more genes that do not occur naturally in the animal, e.g., foreign genes, genetically engineered endogenous genes, etc. The nonnaturally introduced genes, known as transgenes, may be from the same or a different species as the animal but not naturally found in the animal in the configuration and/or at the chromosomal locus conferred by the transgene. Transgenes may comprise foreign DNA sequences, i.e., sequences not normally found in the genome of the host animal. Alternatively or additionally, transgenes may comprise endogenous DNA sequences that are abnormal in that they have been rearranged or mutated in vitro in order to alter the normal in vivo pattern of expression of the gene, or to alter or eliminate the biological activity of an endogenous gene product encoded by the gene. (Watson, J. D., et al., in *Recombinant DNA*, 2d Ed., W.H. Freeman & Co., New York (1992), pages 255–272; Gordon, J. W., *Intl. Rev. Cytol.* 115:171–229 (1989); Jaenisch, R., *Science* 240:1468–1474 (1989); Rossant, *J., Neuron* 2:323–334 (1990)).

The transgenic non-human animals of the invention are produced by introducing transgenes into the germline of the non-human animal. Embryonic target cells at various developmental stages are used to introduce the transgenes of the invention. Different methods are used depending on the stage of development of the embryonic target cell(s).

1. Microinjection of zygotes is the preferred method for incorporating transgenes into animal genomes in the course of practicing the invention. A zygote, a fertilized ovum that has not undergone pronuclei fusion or subsequent cell division, is the preferred target cell for microinjection of transgenic DNA sequences. The murine male pronucleus reaches a size of approximately 20 micrometers in diameter, a feature which allows for the reproducible injection of 1–2 picoliters of a solution containing transgenic DNA sequences. The use of a zygote for introduction of transgenes has the advantage that, in most cases, the injected transgenic DNA sequences will be incorporated into the host animal's genome before the first cell division (Brinster, et al., *Proc. Natl. Acad. Sci.* (*USA*) 82:4438–4442 (1985)). As a consequence, all cells of the resultant transgenic animals (founder animals) stably carry an incorporated transgene at a particular genetic locus, referred to as a transgenic allele. The transgenic allele demonstrates Mendelian inheritance: half of the offspring resulting from the cross of a transgenic animal with a non-transgenic animal will inherit the transgenic allele, in accordance with Mendel's rules of random assortment.

2. Viral integration can also be used to introduce the transgenes of the invention into an animal. The developing embryos are cultured in vitro to the developmental stage known as a blastocyst. At this time, the blastomeres may be infected with appropriate retroviruses (Jaenich, R., *Proc. Natl. Sci.* (*USA*) 73:1260–1264 (1976)). Infection of the blastomeres is enhanced by enzymatic removal of the zona pellucida (Hogan, et al., in *Manipulating the Mouse Embryo*, Cold-Spring Harbor Press, Cold Spring Harbor, N.Y. (1986)). Transgenes are introduced via viral vectors which are typically replication-defective but which remain competent for integration of viral-associated DNA sequences, including transgenic DNA sequences linked to such viral sequences, into the host animal's genome (Jahner, et al., *Proc. Natl. Acad. Sci.* (*USA*) 82:6927–6931 (1985); Van der Putten, et al., *Proc. Natl. Acad. Sci.* (*USA*) 82:6148–6152 (1985)). Transfection is easily and efficiently obtained by culture of blastomeres on a mono-layer of cells producing the transgene-containing viral vector (Van der Putten, et al., *Proc. Natl. Acad. Sci.* (*USA*) 82:6148–6152 (1985); Stewart, et al., *EMBO Journal* 6:383–388 (1987)). Alternatively, infection may be performed at a later stage, such as a blastocoele (Jahner, D., et al., *Nature* 298:623–628 (1982)). In any event, most transgenic founder animals produced by viral integration will be mosaics for the transgenic allele; that is, the transgene is incorporated into only a subset of all the cells that form the transgenic founder animal. Moreover, multiple viral integration events may occur in a single founder animal, generating multiple transgenic alleles which will segregate in future generations of offspring. Introduction of transgenes into germline cells by this method is possible but probably occurs at a low frequency (Jahner, D., et al., *Nature* 298:623–628 (1982)). However, once a transgene has been introduced into germline cells by this method, offspring may be produced in which the transgenic allele is present in all of the animal's cells, i.e., in both somatic and germline cells.

3. Embryonic stem (ES) cells can also serve as target cells for introduction of the transgenes of the invention into animals. ES cells are obtained from pre-implantation embryos that are cultured in vitro (Evans, M. J., et al., *Nature* 292:154–156 (1981); Bradley, M. O., et al., *Nature* 309:255–258 (1984); Gossler, et al., *Proc. Natl. Acad. Sci.* (*USA*) 83:9065–9069 (1986); Robertson et al., *Nature* 322: 445–448 (1986); Robertson, E. J., in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E. J., ed., IRL Press, Oxford (1987), pages 71–112). ES cells, which are commercially available (from, e.g., Genome Systems, Inc., St. Louis, Mo.), can be transformed with one or more transgenes by established methods (Lovell-Badge, R. H., in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E. J., ed., IRL Press, Oxford (1987), pages 153–182). Transformed ES cells can be combined with an animal blastocyst, whereafter the ES cells colonize the embryo and contribute to the germline of the resulting animal, which is a chimera (composed of cells derived from two or more animals) (Jaenisch, R., *Science* 240:1468–1474 (1988); Bradley, A., in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E. J., ed., IRL Press, Oxford (1987), pages 113–151). Again, once a transgene has been introduced into germline cells by this method, offspring may be produced in which the transgenic allele is present in all of the animal's cells, i.e., in both somatic and germline cells.

However it occurs, the initial introduction of a transgene is a Lamarckian (non-Mendelian) event. However, the transgenes of the invention may be stably integrated into germ line cells and transmitted to offspring of the transgenic animal as Mendelian loci. Other transgenic techniques result in mosaic transgenic animals, in which some cells carry the transgenes and other cells do not. In mosaic transgenic animals in which germ line cells do not carry the transgenes, transmission of the transgenes to offspring does not occur. Nevertheless, mosaic transgenic animals are capable of demonstrating phenotypes associated with the transgenes.

Transgenes may be introduced into non-human animals in order to provide animal models for human diseases. Transgenes that result in such animal models include, e.g., transgenes that encode mutant gene products associated with an

inborn error of metabolism in a human genetic disease and transgenes that encode a human factor required to confer susceptibility to a human pathogen (i.e., a bacterium, virus, or other pathogenic microorganism) (Leder et al., U.S. Pat. No. 5,175,383 (Dec. 29, 1992); Kindt et al., U.S. Pat. No. 5,183,949 (Feb. 2, 1993); Small et al., *Cell* 46:13–18 (1986); Hooper et al., *Nature* 326:292–295 (1987); Stacey et al., *Nature* 332:131–136 (1988); Windle et al., *Nature* 343: 665–669 (1990); Katz et al., *Cell* 74:1089–1100 (1993)). Transgenically introduced mutations comprise null ("knock-out") alleles in which a DNA sequence encoding a selectable and/or detectable marker is substituted for a genetic sequence normally endogenous to a non-human animal. Resultant transgenic non-human animals that are predisposed to a disease, or in which the transgene causes a disease, may be used to identify compositions that induce the disease and to evaluate the pathogenic potential of compositions known or suspected to induce the disease (Berns, A. J. M., U.S. Pat. No. 5,174,986 (Dec. 29, 1992)), or to evaluate compositions which may be used to treat the disease or ameliorate the symptoms thereof (Scott et al., WO 94/12627 (1994)).

Offspring that have inherited the transgenes of the invention are distinguished from littermates that have not inherited transgenes by analysis of genetic material from the offspring for the presence of biomolecules that comprise unique sequences corresponding to sequences of, or encoded by, the transgenes of the invention. For example, biological fluids that contain polypeptides uniquely encoded by the selectable marker of the transgenes of the invention may be immunoassayed for the presence of the polypeptides. A more simple and reliable means of identifying transgenic offspring comprises obtaining a tissue sample from an extremity of an animal, e.g., a tail, and analyzing the sample for the presence of nucleic acid sequences corresponding to the DNA sequence of a unique portion or portions of the transgenes of the invention, such as the selectable marker thereof. The presence of such nucleic acid sequences may be determined by, e.g., hybridization ("Southern") analysis with DNA sequences corresponding to unique portions of the transgene, analysis of the products of PCR reactions using DNA sequences in a sample as substrates and oligonucleotides derived from the transgene's DNA sequence, etc.

The present invention is described in further detail in the following non-limiting examples.

EXAMPLE 1

Isolation and Characterization of PCA3 cDNA and Genomic DNA

In order to identify new markers for prostate cancer, a differential display analysis (Liang et al., *Science* 257: 967–971 (1992)) was used to identify genes overexpressed in prostatic carcinomas in comparison to normal prostate; total RNA from normal, benign hyperplastic and malignant prostatic tissue from the same patients was extracted. Using twenty different combinations of primers (four anchored primers, five arbitrary primers), eleven apparently differentially expressed mRNAs were identified (i.e., consistently overexpressed in all carcinomas studied and not expressed in normal or BPH tissue). The complementary DNA (cDNA) fragments were used as probes for Northern blot analysis to confirm the consistent overexpression in the prostatic tumors used for the differential display. One of the probes (named DD3, a 486 bp cDNA) detected two major transcripts (2.3 and 4.0 kb) that are highly overexpressed in 47 of 50 human prostatic tumors studied, whereas no (or very low levels of) expression of these transcripts was found in normal or BPH tissue from the same patients.

To obtain a full length cDNA clone, a cDNA library was constructed using mRNA isolated from human primary prostatic tumor tissue. 250 positive DD3 related clones were obtained from screening this library. 80 clones were purified and the nucleotide sequence of these clones was determined by automated sequence analysis.

A genomic library constructed of human placenta genomic DNA cloned in λFIX2 was screened using DD3 as a probe. Four different clones were obtained, two of them located towards the 5' end of the gene (λFIX-ME3 and -ME4) and two clones located towards the 3' end of the gene (λFIX-ME1 and -ME2). The 5' end of λFIX-ME4 was subcloned and used as a probe to screen the genomic library. Three new, unique clones were isolated (λFIX-IH1, IH2, and IH6).

From the 80 analyzed cDNA clones, at least four different transcripts were shown to be present due to alternative splicing or alternative polyadenylation. Sequence analysis of the genomic clones as compared to the cDNA clones revealed the genomic structure of the PCA3 gene. Three introns and 4 exons are present. The first intron is approximately 20 kb in length.

The first cDNA species is found in approximately 5% of the cDNA clones and contains exons 1, 2, 3, 4a and 4b (poly-adenylation after 4b is preceeded by a real consensus poly-A-addition signal)(FIG. 1).

The second cDNA species, found in approximately 15% of the cDNA cloned, contains exons 1, 3, 4a, 4b and 4c, arises by alternative splicing of the second exon (not present in this cDNA) and terminates at a different (real consensus) poly-A-addition signal (FIG. 1).

The third cDNA species contains exons 1, 3, 4a, and 4b and is the most common one found (approximately 65% of 80 clones) (FIG. 1). This cDNA is most likely responsible for the most prominent transcript seen by Northern blot analysis (2 kb).

The fourth cDNA species detected contains exons 1, 3, and 4a representing about 15% of clones, and terminates after 4a, which is the original DD3 clone stop site (FIG. 1). The poly-A-addition signal present here is close to the consensus sequence.

PCA3 is a gene wherein significant alternative splicing (as well as alternative poly-adenylation) occurs, as evidenced by the differently sized transcripts observed on Northern blots and the different types of clones identified. As mentioned previously, other splicing variants can be identified, as virtually every combination of exons is possible. For instance, a cDNA clone having exons 2, 3, 4a, 4b, and 4c has recently been identified. Indeed, it appears that clones representing virtually all possible exon combinations have been isolated.

One such splicing variant was identified by sequencing a clone named λDD3.6. λDD3.6 is a λgt11 clone identified and isolated upon screening of a cDNA library made from prostate RNA of a 25 year old male (obtained from Clonetech) with a PCA3 probe. λDD3.6 contains exon 3, 4a, 4b, 4c, and 4d. However, this cDNA clone also contains intron sequences (part of intron 2, as well as intron 3).

A comparison of the two deposited clones PMB9 and λDD3.6 is shown in FIG. 3.

Different combinations of exons were examined by computer analysis to identify open reading frames (ORFs) and to predict the protein encoding region. The longest ORF was

also the most highly likely protein encoding region. The longest ORF of 153 nucleotides encodes a small peptide of 51 amino acids, PCA3. PCA3 is encoded by part of exon 3 and 4a. The small size of the protein suggests that the protein most likely functions as a messenger molecule and has the potential to be secreted from the cells. The nucleotide sequence of exons 1–4a–d and the amino acid sequence of PCA3 are shown in FIG. 2 and FIG. 5 (SEQ ID NO:1 and 6 and 2 and 7, respectively).

It will be recognized by the person of ordinary skill, that a cDNA clone comprising the nucleic acid sequence presented in SEQ ID NO:6 and shown in FIG. 5 can be obtained as previously described by isolating and characterizing PCA3 cDNA clones. For example, and as commonly known in the art, probes which are specific to at least one of the 5' end, exon 1, 2, 3, 4a, 4b, 4c and 4d can be further used to increase the probability of having a full-length PCA3 cDNA clone. 96-well plates, for example, can be used to screen a large number of PCA3 positive cDNA clones, using the probes mentioned above. Of course, PCA3 positive clones can also be sequenced, as commonly known and as described herein, until a desired cDNA clone is obtained.

In addition it is also possible to obtain a cDNA clone comprising the sequence shown in SEQ ID NO:6 and shown in FIG. 5, using PCA3 specific primers and an amplifying method such as PCR. For example, PCR technology with primers specific for the ultimate 5' and 3' end of the PCA3 cDNA, could be used to amplify a desired product (almost 4 kb) from RNA, isolated for example from prostatic tumors, and clone the PCR products. However, since PCR amplification may introduce mistakes, a sequencing of the complete cDNA would most likely be required.

As well known to the person of ordinary skill, a cDNA clone comprising the sequence shown in SEQ ID NO:6 and shown in FIG. 5 can also be constructed using the clones described herein (or newly isolated ones) and conventional genetic engineering methods.

For example, such a full length cDNA clone can be constructed using the deposited clones pMB9 and λDD3.6. A non-limiting example of a strategy to construct such a cDNA clone comprising the nucleic acid sequence of SEQ ID NO:6 and FIG. 5, is described below.

λDD3.6 phage DNA is digested to completion with NdeI, and the approximately 2 kb NdeI fragment isolated from an agarose gel. This fragment contains part of PCA3 exon 4b, exons 4c+4d and about 50 nucleotides of phage DNA. The ends of this 2 kb fragment are then filled in with Klenow-fragment DNA polymerase and dNTPs, the blunt-ended fragment are then ligated into the HincII/SmaI sites of Bluescript SK. The loss of the HindIII site of Bluescript by the HincII and SmaI digestion is essential for further cloning steps in this particular strategy (see below). It is to be noted that NdeI sites are also present in phage λgt11, giving rise to several additional fragments, some of which are close to 2 kb (i.e., a 1.8 kb and a 2.5 kb fragment). Nevertheless, it is straight forward to separate these different bands on an agarose gel. The correct orientation of insertion of the blunt-ended 2 kb NdeI fragment of λDD3.6 into Bluescript (termed construct PCA3-X) can be verified by a single SacI digestion, which should yield a ~0.45 and ~4.5 kb fragment by Ethidium-bromide staining of agarose gels. Sequence analysis may be performed to confirm the identity of the PCA3 insert.

The PCA3-X construct is then digested to completion with HindIII and BamHI and a 4.8 kb vector-insert isolated from an agarose gel. This results in the removal of ~0.2 kb of DNA from the insert. pMB9 can be simultaneously digested to completion with BamHI and HindIII and the 1.9 kb insert (containing PCA3 exons 1, 2, 3, 4a and most of exon 4b) isolated from an agarose gel. The pMB9-derived insert is ligated into the BamHI/HindIII site of the PCA3-X construct. The resulting construct, PCA3-Y contains the complete cDNA of PCA3, except for the first 22 nucleotides of exon1 (see below and FIG. 4). These 22 nucleotides can be added to the PCA3 cDNA by cutting the PCA3-Y construct and the oligo-(74)-mer (SEQ ID NO:8) to completion with BamHI and PstI and ligating the oligomer in construct PCA3-Y, resulting in construct PCA3-Z. Nucleotide sequence analysis can be performed to verify that the oligo was properly ligated (i.e. to confirm that just one oligo was ligated and not a whole array of oligos). Of course, a sequencing of the resulting cDNA in PCA3-Z, can be performed to verify the integrity of the nucleic acid sequence.

Screening of a somatic cell hybrid panel revealed that the gene encoding PCA3 is located on human chromosome 9. Using a mixture of four PCA3-related genomic clones as a probe to hybridize to metaphase chromosomes of human lymphocytes, PCA3 was mapped to 9q21–22 (See also, FIG. 1).

The conservation of PCA3 gene during evolution was studied by Southern blot analysis and revealed that a homolog of this gene is present in monkey, cow, horse, sheep, goat and pig. The gene is also present in dog and cat. By comparison, the gene encoding PSA is only found in primates.

EXAMPLE 2

Prostate Specific Expression of PCA3

Upon developing PCA3 specific primers, RT-PCR analysis was performed using RNA from several normal human tissues. At 40 cycles of PCR, PCA3 related products in normal prostate and BPH tissues were amplified. PCA3 expression is very prostate specific since no PCA3 product could be amplified under these conditions in the following normal human tissues: artery, brain, breast, bladder, colon, duodenum, heart, liver, lung, ovary, pancreas, placenta, seminal vesicles, skeletal muscle, skin, spinal cord, spleen and testis. Also in the human prostate cancer cell lines ALVA-31, DU145, JCA-1, PPC-1, PC3, and TSU-Pr1 no PCA3 related PCR product could be detected. In the cell line LNCaP a product can be obtained after 40 cycles of PCR (whereas under the same conditions a product can be obtained in prostatic tumors within 20 cycles). The technology used to assess the prostate specific expression of PCA3 can be adapted in a diagnostic test for prostate cancer. In addition, it can be adapted to the identification of the prostatic origin of a metastase.

Furthermore, a semi-quantitative RT-PCR analysis to compare the expression of PCA3 to that of PSA (prostate-specific antigen) and PSM (prostate-specific membrane antigen) and to establish if PCA3-RT-PCR analysis can be used to distinguish malignant from benign prostatic specimens was performed. After quantification of the RT-reaction, 10 ng of cDNA was used for the PCR reaction and as a control, beta-2 microglobulin was also examined. PCA3 products found allowed a clear distinction between benign and malignant specimens in 23 of 25 cases studied whereas PSA and PSM could not make this distinction: approximately equal

amounts of product were found in normal and tumor samples. The expression of PSA and PCA3 was also compared by Northern blot analysis, which clearly shows the higher tumor-specificity of PCA3. At least a 20-fold overexpression of PCA3 in prostatic carcinomas as compared to normal or BPH tissues is observed. This is distinctly different from expression of PSM and PSA, both of which are decreased in malignant versus benign tissues. Thus, PCA3 appears to be a good marker for diagnostic of prostate cancer.

An ideal tumor marker for prostate cancer should not only be able to positively distinguish between benign and malignant tissues but also be able to predict clinical outcome (cure or progression) of patients afflicted with this disease. Data has shown that indeed, the level of expression of PCA3 tends to be positively correlated with tumor grade.

RISH (and eventually immunohistochemistry) is used to establish whether or not there is correlation beween overexpression of PCA3, tumor grade, stage, and clinical outcome. For both the paraffin-embedded and frozen specimens, long-term clinical follow-up is available. Using computer-assisted image analysis, quantitation of PCA3 expression levels as detected by RISH is performed and this is normalized to an external reference (Tamimi et al., *Cancer Res.* 53: 5512–16 (1993); Tamimi et al., *B.J. Cancer* (1996)). A multivariate regression analysis including Gleason: grade, pathological tumor stage, clinical tumor stage, PSA levels and PCA3 expression will be used to establish whether PCA3 is an accurate predictor of progression and has (additional) prognostic value.

Reverse transcriptase polymerase chain reaction (RT-PCR) assays have been developed to detect occult hematogenous micrometastatic cells that might otherwise have gone undetected by presently available staging modalities. Such RT-PCR assays have already been performed in patients with prostate cancer and other malignancies. A highly sensitive (nested) RT-PCR assay (or other types of amplification assays including without being limited to NASBA, PCR, QB rep., SOA, TMA, and LCR (Winn-Deen, *J. Clin., Liquid Assay* 19: 21–26 (1996)) can be used to detect prostate cancer cells in the circulating blood of prostate: cancer patients to identify patients at risk for having or developing metastases. Experiments will include appropriate controls (e.g. β-2-microglobulin) and will be performed in a semi-quantitative way (i.e., quantify the cDNA synthesis and use equal amounts of input for the PCR analysis).

The molecular staging studies will be performed in the larger context of the BIOMED II program (*Markers for Prostate Cancer*). In this extensive collaborative study, PSA and PSM will be studied as well as other potentially interesting markers for prostate cancer. Blood samples are already being collected from patients that are diagnosed with prostatic disease in the participating institutions. An optimization of the collecting and handling of blood samples from patients for the detection of circulating tumor cells has been initiated. The use for example of vacutainer™ CPT-tubes (BecktonDickinson) for blood collection and purification of peripheral blood leukocytes in combination with a Trizol™ RNA-extraction procedure (guanidinium thiocyanate based) resulted in the preparation of RNA qualitatively and quantitatively suitable for PCR analysis. The use of PCA3-specific primers, to amplify PCA3 transcripts in RNA extracted from blood from prostate cancer patients, revealed that the presence of prostate cancer cells in the blood circulation, of not only patients with proven metastases, but also of patients with assumed localized disease, could be detected. More extensive studies on a larger patient population and a correlation with clinical data and follow-up will be carried out to determine the prognostic value of PCA3 for the individual prostate cancer patient.

Nested RT-PCR analysis (or similar amplification methods) should prove instrumental in determining whether there are any organs (not yet tested) that express PCA3. For example, Cowper's gland (same embryonic origin as the prostate) and also skene's gland (female "homologue" to the prostate) will be tested for PCA3.

In one "normal" prostate tissue specimen that contained 10% of tumor cells, PCA3 expression was detected indicating the high sensitivity of PCA3 as a tumor marker. In this manner, PCA3 expression was also detected in a few BPH samples that were subsequently found to contain small areas of tumor cells. The level of expression of PCA3 in prostatic cancers shows a trend towards a positive correlation with tumor grade. These data are based on analysis of autoradiographs resulting from Northern blot hybridization.

The observation that PCA3 expression seems to increase with loss of differentiation is different from what is reported for PSA, since PSA expression levels decrease with loss of differentiation (Hakalahti et al., *Int. J. Cancer* 55:590–597 (1993)). There is at least 20-fold overexpression of PCA3 in prostatic carcinomas in comparison to normal or BPH tissues. This is distinctly different from the expression of PSA which is reported to decrease in malignant versus benign tissues. PCA3 expression was detected in 4 of 4 metastases studied.

EXAMPLE 3

Identification of a Transcription Start Site of PCA3

In order to determine the transcription start site of PCA3 primer extension analysis, S1-nuclease mapping and 5'RACE (rapid amplification of cDNA ends) assays were performed. The major transcription start site was found to be located within a range of 4 nucleotides (FIG. 4).

The results of these experiments further lengthen the size of the cDNA in a 5' direction by a further 22 nt with respect to the cDNA sequence of pMB9 (SEQ ID NO:1 and FIG. 2). This additional 5' polynucleotide sequence is also shown in SEQ ID NO:6 and FIG. 5).

All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

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ggt 123

What is claimed is:

1. An isolated nucleic acid molecule comprising a polynucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence comprising the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 682.97;

(b) a nucleotide sequence comprising the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 100521;

(c) a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO:1, 3, 4 or 6, wherein said nucleotide sequence is over-expressed in prostate cancer tissue as compared to normal human tissues;

(d) a nucleotide sequence fully complementary to any of the nucleotide sequences in (a), (b) or (c); and

(e) a probe or primer that hybridizes under high stringency conditions to any of the nucleotide sequences in (a), (b), (c) or (d), said high stringency conditions comprising a hybridization at 68° C. in 5×SSC, 5× Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA, wherein said probe or primer does not hybridize to nucleotides 511–985 of SEQ ID NO:1, nucleotides 346–820 of SEQ ID NO:3, nucleotides 346–820 of SEQ ID NO:4, or nucleotides 533–1007 of SEQ ID NO:6, and wherein said probe or primer selectively hybridizes to polynucleotides over-expressed in prostate cancer tissue as compared to normal human tissues selected from the group consisting of: artery, brain, breast, duodenum, heart, liver, ovary, placenta, seminal vesicles, skeletal muscle, skin, spinal cord, spleen and testis.

2. An isolated nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:1, 3, 4 or 6.

3. The isolated nucleic acid molecule according to claim 2, wherein the molecule encodes the polypeptide comprising the complete amino acid sequence set forth in SEQ ID NO:2 or 7.

4. The isolated nucleic acid molecule according to claim 1, wherein the nucleotide sequence encodes a PCA3 polypeptide comprising the complete amino acid sequence encoded by the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 682.97.

5. The isolated nucleic acid molecule according to claim 1, wherein the nucleotide sequence encodes a PCA3 polypeptide comprising the complete amino acid sequence

encoded by the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 100521.

6. An isolated nucleic acid molecule consisting of 10 to 50 nucleotides which specifically hybridizes to PCA3 RNA or DNA, wherein said nucleic acid molecule is, or is complementary to, a nucleotide sequence consisting of at least 10 consecutive nucleotides from PCA3 exon 1 (1–98 of SEQ ID NO:1 or 1–120 of SEQ ID NO:6), 2 (99–263 of SEQ ID NO:1 or 121–285 of SEQ ID NO:6), 3 (264–446 of SEQ ID NO:1 or 286–468 of SEQ ID NO:6), 4a (447–985 of SEQ ID NO:1 or 469–1007 of SEQ ID NO:6), 4b (986–2037 of SEQ ID NO:1 or 1008–2066 of SEQ ID NO:6), 4c (2067–2622 of SEQ ID NO:6), or 4d (2623–3582 of SEQ ID NO:6), and wherein said nucleic acid molecule does not specifically hybridize to nucleotides 511–985 of SEQ ID NO:1 or nucleotides 533–1007 of SEQ ID NO:6.

7. A method of detecting PCA3 nucleic acid in a sample comprising:

a) contacting said sample with the nucleic acid molecule according to claim 6 under conditions such that hybridization occurs; and

b) detecting the presence of said molecule bound to PCA3 nucleic acid.

8. A kit for detecting the presence of PCA3 nucleic acid in a sample comprising at least one container means having disposed therein the nucleic acid molecule according to claim 6.

9. A recombinant nucleic acid molecule comprising a vector and the nucleic acid molecule according to claim 1.

10. An isolated cell that contains the recombinant nucleic acid molecule according to claim 9.

11. A recombinant nucleic acid molecule comprising a vector and the nucleic acid molecule according to claim 2.

12. An isolated cell that contains the recombinant nucleic acid molecule according to claim 11.

13. An isolated nucleic acid molecule comprising the polynucleotide sequence set forth from nucleotides 401 to 553 of SEQ ID NO:6.

14. The isolated nucleic acid molecule of claim 13, wherein said polynucleotide sequence encodes the complete amino acid sequence as set forth in SEQ ID NO:7.

15. The isolated nucleic acid molecule according to claim 2, wherein the molecule comprises the nucleotide sequence as set forth in SEQ ID NO: 6.

16. The isolated nucleic acid molecule according to claim 2, wherein the molecule comprises the nucleotide sequence as set forth in SEQ ID NO:1.

17. The isolated nucleic acid molecule according to claim 2, wherein the molecule comprises the nucleotide sequence as set forth in SEQ ID NO:3.

18. The isolated nucleic acid molecule according to claim 2, wherein the molecule comprises the nucleotide sequence as set forth in SEQ ID NO:4.

19. The recombinant nucleic acid molecule according to claim 11, wherein the molecule comprises the nucleotide sequence as set forth in SEQ ID NO:6.

20. A recombinant nucleic acid molecule comprising a vector and the nucleic acid molecule according to claim 13.

21. An isolated cell that contains the recombinant nucleic acid molecule according to claim 20.

22. An isolated nucleic acid molecule comprising a polynucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence comprising the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 682.97;

(b) a nucleotide sequence comprising the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 100521;

(c) a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO:1, 3, 4 or 6, wherein said nucleotide sequence is over-expressed in prostate cancer tissue as compared to normal human tissues;

(d) a nucleotide sequence fully complementary to any of the nucleotide sequences in (a), (b) or (c); and

(e) a probe or primer that hybridizes under high stringency conditions to any of the nucleotide sequences in (a), (b), (c) or (d), said high stringency conditions comprising a hybridization at 68° C. in 5×SSC, 5× Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA, wherein said probe or primer does not hybridize to nucleotides 511–985 of SEQ ID NO:1, nucleotides 346–820 of SEQ ID NO:3, nucleotides 346–820 of SEQ ID NO:4, or nucleotides 533–1007 of SEQ ID NO:6, and wherein said probe or primer selectively hybridizes to polynucleotides over-expressed in prostate cancer tissue as compared to human prostate cancer cell lines selected from the group consisting of: ALVA-31, JCA-1 and PPC-1.

23. A recombinant nucleic acid molecule comprising a vector and the nucleic acid molecule according to claim 22.

24. An isolated cell that contains the recombinant nucleic acid molecule according to claim 23.

25. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence in (e) is a nucleic acid sequence molecule consisting of 10 to 50 nucleotides.

26. The isolated nucleic acid molecule of claim 22, wherein the nucleotide sequence in (e) is a nucleic acid sequence molecule consisting of 10 to 50 nucleotides.

27. An isolated nucleic acid molecule comprising a polynucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence comprising the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 682.97;

(b) a nucleotide sequence comprising the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 100521;

(c) a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO:1, 3, 4 or 6, wherein said nucleotide sequence is over-expressed in prostate cancer tissue as compared to normal human tissues;

(d) a nucleotide sequence fully complementary to any of the nucleotide sequences in (a), (b) or (c); and

(e) a probe or primer that hybridizes under high stringency conditions to any of the nucleotide sequences in (a), (b), (c) or (d), said high stringency conditions comprising a hybridization at 68° C. in 5×SSC, 5× Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA, wherein said probe or primer selectively hybridizes to polynucleotides over-expressed in prostate cancer tissue as compared to normal human tissue.

28. The isolated nucleic acid molecule according to claim 27, wherein the nucleotide sequence encodes a PCA3 polypeptide comprising the complete amino acid sequence encoded by the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 682.97 or as accession number CBS 100521.

29. An isolated nucleic acid molecule consisting of 10 to 50 nucleotides which specifically hybridizes to PCA3 RNA or DNA, wherein said nucleic acid molecule is, or is complementary to, a nucleotide sequence consisting of at least 10 consecutive nucleotides from PCA3 exon 1 (1–98 of

SEQ ID NO:1 or 1–120 of SEQ ID NO:6), 2 (99–263 of SEQ ID NO:1 or 121–285 of SEQ ID NO:6), 3 (264–446 of SEQ ID NO:1 or 286–468 of SEQ ID NO:6), 4a (447–985 of SEQ ID NO: 1 or 469–1007 of SEQ ID NO:6), 4b (986–2037 of SEQ ID NO:1 or 1008–2066 of SEQ ID NO:6), 4c (2067–2622 of SEQ ID NO:6), or 4d (2623–3582 of SEQ ID NO:6).

30. A method of detecting PCA3 nucleic acid in a sample comprising:

a) contacting said sample with the nucleic acid molecule according to claim 29 under conditions such that hybridization occurs; and

b) detecting the presence of said molecule bound to PCA3 nucleic acid.

31. A kit for detecting the presence of PCA3 nucleic acid in a sample comprising at least one container means having disposed therein the nucleic acid molecule according to claim 29.

32. A recombinant nucleic acid molecule comprising a vector and the nucleic acid molecule according to claim 27.

33. An isolated cell that contains the recombinant nucleic acid molecule according to claim 32.

34. An isolated nucleic acid molecule comprising a polynucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence comprising the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 682.97;

(b) a nucleotide sequence comprising the polynucleotide clone contained in the deposit at the Centraal voor Schimmelcultures as accession number CBS 100521;

(c) a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO:1, 3, 4 or 6, wherein said nucleotide sequence is over-expressed in prostate cancer tissue as compared to normal human tissues;

(d) a nucleotide sequence fully complementary to any of the nucleotide sequences in (a), (b) or (c); and

(e) a probe or primer that hybridizes under high stringency conditions to any of the nucleotide sequences in (a), (b), (c) or (d), said high stringency conditions comprising a hybridization at 68° C. in 5×SSC, 5× Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA, wherein said probe or primer selectively hybridizes to polynucleotides over-expressed in prostate cancer tissue as compared to human prostate cancer cell lines selected from the group consisting of: ALVA-31, DU145, JCA-1, PPC-1, PC3, and TSU-Pr1.

35. A recombinant nucleic acid molecule comprising a vector and the nucleic acid molecule according to claim 34.

36. An isolated cell that contains the recombinant nucleic acid molecule according to claim 35.

37. The isolated nucleic acid molecule of claim 27, wherein the nucleotide sequence in (e) is a nucleic acid sequence-molecule consisting of 10 to 50 nucleotides.

38. The isolated nucleic acid molecule of claim 34, wherein the nucleotide sequence in (e) is a nucleic acid sequence molecule consisting of 10 to 50 nucleotides.

39. The isolated nucleic acid molecule of claim 27, wherein the normal tissue in (e) is selected from the group consisting of: artery, brain, breast, bladder, colon, duodenum, heart, liver, lung, ovary, pancreas, placenta, seminal vesicles, skeletal muscle, skin, spinal cord, spleen and testis.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

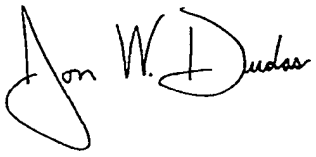
PATENT NO. : 7,008,765 B1
APPLICATION NO. : 09/402713
DATED : March 7, 2006
INVENTOR(S) : Bussemakers et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title page, column 1, Item (73), please delete "The Johns Hopkins University, Baltimore, MD (US); Stichting Katholieke Universiteit more particularly The University Medical Centre, Nijmegen (NL)" and insert therein -- The Johns Hopkins University, Baltimore, MD (US); Stichting Katholieke Universiteit, more particularly The University Medical Centre Nijmegen, Nijmegen (NL) --.

Signed and Sealed this

Twenty-fifth Day of March, 2008



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CERTIFICATE OF CORRECTION

Exhibit 2

PATENT NO. : 7,008,765 B1
APPLICATION NO. : 09/402713
DATED : March 7, 2006
INVENTOR(S) : Bussemakers et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title page, column 1, Item (73), please delete "The Johns Hopkins University, Baltimore, MD (US); Stichting Katholieke Universiteit more particularly The University Medical Centre, Nijmegen (NL)" and insert therein -- The Johns Hopkins University, Baltimore, MD (US); Stichting Katholieke Universiteit, more particularly The University Medical Centre Nijmegen, Nijmegen (NL) --.

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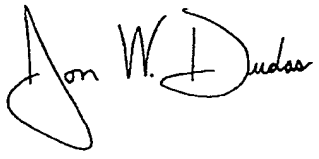

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Exhibit 3

Patent Bibliographic Data					03/27/2012 01:29 PM
Patent Number:	7008765		**Application Number:**	09402713	
Issue Date:	03/07/2006		**Filing Date:**	06/13/2000	
Title:	PCA3, PCA3 GENES, AND METHODS OF USE				
Status:	8th year fee window opens: 03/07/2013			**Entity:**	Large
Window Opens:	03/07/2013	**Surcharge Date:**	09/10/2013	**Expiration:**	N/A
Fee Amt Due:	Window not open	**Surchg Amt Due:**	Window not open	**Total Amt Due:**	Window not open
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Most recent events (up to 7):	08/20/2009 08/20/2009 08/19/2009	Payor Number Assigned. Payer Number De-assigned. Payment of Maintenance Fee, 4th Year, Large Entity. --- End of Maintenance History ---			
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RECORDATION DATE: 06/13/2000

REEL/FRAME: 010902/0386
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
BUSSEMAKERS, MARION J.G

DOC DATE: 05/30/2000

ASSIGNEE:
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GEERT GROOTEPLEIN 10
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6500 HB NIJMEGEN, NETHERLANDS

SERIAL NUMBER: 09402713
PATENT NUMBER:

FILING DATE: 06/13/2000
ISSUE DATE:

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ASSIGNMENT

In consideration of the sum of One Dollar ($1.00) or equivalent and other good and valuable consideration paid to each of the undersigned inventor(s): Marion J. C. Bussemakers, the undersigned inventor(s) hereby sell(s) and assign(s) to UNIVERSITY HOSPITAL NIJMEGEN (the Assignee) his/her entire right, title and interest, including the right to sue for past infringement and to collect for all past, present and future damages:

check applicable box(es) ☒ for the United States of America (as defined in 35 U.S.C. § 100),
☒ and throughout the world,

(a) in the invention(s) known as PCA3, PCA3 Genes, and Methods of Use for which application(s) for patent in the United States of America has (have) been executed by the undersigned on May 30, 2000 (also known as United States Application No. (U.S. National Phase of International Appl. No. PCT/CA98/00346; U.S. Appl. No. 09/402,713), filed (International Filing Date: April 9, 1998), in any and all applications thereon, in any and all Letters Patent(s) therefor, and

(b) in any and all applications that claim the benefit of the patent application listed above in part (a), including continuing applications, reissues, extensions, renewals and reexaminations of the patent application or Letters Patent therefor listed above in part (a), to the full extent of the term or terms for which Letters Patents issue, and

(c) in any and all inventions described in the patent application listed above in part (a), and in any and all forms of intellectual and industrial property protection derivable from such patent application, and that are derivable from any and all continuing applications, reissues, extensions, renewals and reexaminations of such patent application, including, without limitation, patents, applications, utility models, inventor's certificates, and designs together with the right to file applications therefor; and including the right to claim the same priority rights from any previously filed applications under the International Agreement for the Protection of Industrial Property, or any other international agreement, or the domestic laws of the country in which any such application is filed, as may be applicable;

all such rights, title and interest to be held and enjoyed by the above-named Assignee, its successors, legal representatives and assigns to the same extent as all such rights, title and interest would have been held and enjoyed by the Assignor had this assignment and sale not been made.

The undersigned inventor(s) agree(s) to execute all papers necessary in connection with the application(s) and any continuing (continuation, divisional, or continuation-in-part), reissue, reexamination or corresponding application(s) thereof and also to execute separate assignments in connection with such application(s) as the Assignee may deem necessary or expedient.

The undersigned inventor(s) agree(s) to execute all papers necessary in connection with any interference or patent enforcement action (judicial or otherwise) related to the application(s) or any continuing (continuation, divisional, or continuation-in-part), reissue or reexamination application(s) thereof and to cooperate with the Assignee in every way possible in obtaining evidence and going forward with such interference or patent enforcement action.

The undersigned inventor(s) hereby represent(s) that he/she has full right to convey the entire interest herein assigned, and that he/she has not executed, and will not execute, any agreement in conflict therewith.

The undersigned inventor(s) hereby grant(s) Robert Greene Sterne, Esquire, Registration No. 28,912; Edward J. Kessler, Esquire, Registration No. 25,688; Jorge A. Goldstein, Esquire, Registration No. 29,021; Samuel L. Fox, Esquire, Registration No. 30,351; David K.S. Cornwell, Esquire, Registration No. 31,944; Robert W. Esmond, Esquire, Registration No. 32,893; Tracy-Gene G. Durkin, Esquire, Registration No. 32,831; Michele A. Cimbala, Esquire, Registration No. 33,851; Michael B. Ray, Esquire, Registration No. 33,997; Robert E. Sokohl, Esquire Registration No. 36,013; Eric K. Steffe, Esquire, Registration No. 36,688; Michael Q. Lee, Esquire, Registration No. 35,239; Steven R. Ludwig, Esquire, Registration No. 36,203; Raz E. Fleshner, Esquire Registration No 34,331; John M. Covert, Esquire, Registration No. 38,759; and Linda E. Alcorn, Esquire, Registration No. 39,588; all of STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C., 1100 New York Avenue, N.W., Suite 600, Washington, D.C. 20005-3934, power to insert in this assignment any further identification that may be necessary or desirable in order to comply with the rules of the United States Patent and Trademark Office for recordation of this document.

IN WITNESS WHEREOF, executed by the undersigned inventor(s) on the date opposite his/her name.

Date: May 30, 2000 Signature of Inventor: _____

Marion J. G. Bussemakers

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Exhibit 5

500005191A

AUGUST 23, 2004

PTAS

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C
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WASHINGTON, DC 20005

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RECORDATION DATE: 08/23/2004 REEL/FRAME: 015020/0591
NUMBER OF PAGES: 13

BRIEF: CHANGE OF NAME (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
UNIVERSITY HOSPITAL NIJMEGEN DOC DATE: 11/01/1999

ASSIGNEE:
UNIVERSITY MEDICAL CENTRE NIJMEGEN
GEERT GROOTEPLEIN 10
P.O. BOX 9101
NIJMEGAN, NETHERLANDS 6500 HB

SERIAL NUMBER: 09402713 FILING DATE: 06/13/2000
PATENT NUMBER: ISSUE DATE:
TITLE: PCA3, PCA3 GENES, AND METHODS OF USE

015020/0591 PAGE 2

VIOLET MCCOY, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

UMC St Radboud

STATEMENT

Herewith we declare that:

- d.d. November 1st, 1999, the **Faculty of Medicine of the University of Nijmegen** integrated with the **University Hospital Nijmegen;**
- d.d. November 1st, 1999, these two entities together became one entity named the **University Medical Centre Nijmegen,** which is part of **Stichting Katholieke Universiteit,** a legal entity (foundation) by virtue of the Dutch 'Wet op het hoger onderwijs en wetenschappelijk onderzoek' (Higher Education and Academic Research Act) according to Dutch law.

IN WITNESS WHEREOF, the University Medical Centre Nijmegen hereto has executed this Statement by her duly authorized representatives,

Nijmegen, 26.05.2004

Prof.dr. C.L.A. van Herwaarden
Chairman of the Executive Board

Nijmegen, 26.05.2004

mr. A.A.M. Kerckhoffs
Secretary of the Executive Board

KAMER VAN KOOPHANDEL
CENTRAAL GELDERLAND

File number: 41055629 Page 00001

English translation of an extract from the commercial register of the Chamber of Commerce and Industries for Centraal Gelderland

---

Legal person:

Legal form	:Foundation
Corporate name	:Stichting Katholieke Universiteit
Abbreviated name	:Stichting K.U.
Statutory seat	:Nijmegen
Address	:Geert Grooteplein-Noord 9, 6525EZ Nijmegen
Mailing address	:Postbus 9102, 6500HC Nijmegen
Telephone numbers	:024-3611555 / 024-3615444
Fax number	:024-3541468
Incorporation deed	:30-07-1905
Deed of latest amendment of articles	:27-10-1997
Business activities suspended as at	:01-03-2004

Director(s):

Name	:Grobbeé, Johannes Jacobus Jozef
Date and place of birth	:19-03-1940, Dalfsen
Address	:Pothoofd 116, 7411ZD Deventer
Date of entry into office	:27-03-1995
Powers	:Authorised jointly (with other directors, see articles)
Date of (present) representative authority	:27-03-1995

Name	:Lockefeer, Henricus Antonius Ludovicus
Date and place of birth	:23-10-1938, Roosendaal en Nispen
Address	:Jacob Obrechtlaan 13, 1401CE Bussum
Date of entry into office	:05-04-1995
Title	:Vice-voorzitter
Powers	:Solely/independently authorised
Date of (present) representative authority	:20-12-2002

Name	:Brentjens, Josephus Lambertus
Date and place of birth	:30-03-1940, Haelen
Address	:Koepellaan 6, 2061CV Bloemendaal
Date of entry into office	:11-03-1993
Title	:Voorzitter

24,00 04-05-2004 Page 00002 follows.

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KAMER VAN KOOPHANDEL
CENTRAAL GELDERLAND

Powers	:Solely/independently authorised
Date of (present) representative authority	:19-10-2001
Name	:Splinter-van Kan, Hubertina Anna Gertruda
Date and place of birth	:09-06-1947, Maastricht
Address	:Prinsengracht 698, 1017LA Amsterdam
Date of entry into office	:11-03-1993
Powers	:Authorised jointly (with other directors, see articles)
Date of (present) representative authority	:11-03-1993
Name	:de Wijkerslooth de Weerdesteijn, Roelof Jozef
Date and place of birth	:18-10-1946, 's-Gravenhage
Address	:Theresiaweg 2, 6523ND Nijmegen
Date of entry into office	:01-05-2000
Title	:Voorzitter van het college van bestuur
Powers	:Solely/independently authorised
Date of (present) representative authority	:01-05-2000
Name	:Vugts, Johannes Franciscus Theresia
Date and place of birth	:10-07-1942, 's-Gravenhage
Address	:Bergweg 24, 6523MD Nijmegen
Date of entry into office	:01-01-2003
Powers	:Authorised jointly (with other directors, see articles)
Date of (present) representative authority	:01-01-2003
Name	:Wiggers-Rust, Liduina Francisca
Date and place of birth	:12-10-1951, Nijmegen
Address	:Burg.de Millylaan 8, 7231DR Warnsveld
Date of entry into office	:01-09-2002
Powers	:Authorised jointly (with other directors, see articles)
Date of (present) representative authority	:01-09-2002
Name	:van Herwaarden, Cornelis Lambertus August
Date and place of birth	:25-01-1944, Nijmegen
Address	:Kwakkenbergweg 57, 6523ML Nijmegen

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Date of entry into office	:01-12-2003
Title	:UMC: voorzitter raad van bestuur
Powers	:Solely/independently authorised
Date of (present) representative authority	:01-12-2003
Name	:Ruiter, Dirk Jacob
Date and place of birth	:26-06-1947, Harlingen
Address	:Vinkenlaan 14, 6581CK Malden
Date of entry into office	:01-01-2004
Title	:UMC: vice voorzitter raad van bestuur/ decaan
Powers	:Solely/independently authorised
Date of (present) representative authority	:01-01-2004

Authorized signatory(signatories):

Name	:Hegeman, Wilhelmus Everhardus Joseph
Date of birth	:20-03-1948
Address	:Violenstraat 32, 6602CL Wijchen
Date of entry into office	:30-01-1995
Title	:UMC: strategisch inkoper
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:30-01-1995
Name	:de Bekker, Michaël Johannes Jozef
Date and place of birth	:21-07-1957, Lith
Address	:Deken Fritsenstraat 71, 5243VM Rosmalen
Date of entry into office	:01-05-1992
Title	:UMC: Directeur financieel economische zaken
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-05-1992
Name	:Hesseling, Jozef Hendrik
Date and place of birth	:14-02-1946, Arnhem
Address	:Zevendreef 3034, 6605VC Wijchen
Date of entry into office	:01-11-1991
Title	:UMC: strategisch inkoper
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-11-1991

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KAMER VAN KOOPHANDEL
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File number: 41055629 Page 00004

Name	:Kerckhoffs, Aemilius Antonius Maria
Date and place of birth	:11-10-1949, Nijmegen
Address	:Jaspisstraat 3, 6534SE Nijmegen
Date of entry into office	:01-11-1991
Title	:UMC: secretaris Raad van Bestuur
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-11-1991

Name	:Schils, Denis Antoon Gerard Jérôme
Date and place of birth	:15-10-1940, Roosendaal en Nispen
Address	:Gildestraat 39, 6691DT Gendt
Date of entry into office	:01-10-1993
Title	:Secretaris stg kun
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-10-1993

Name	:van Baal, Felix Marcus Jacobus Maria
Date and place of birth	:21-10-1957, Nijmegen
Address	:Witte Hoeflaan 24, 5343EH Oss
Date of entry into office	:30-01-1995
Title	:UMC: Algemeen directeur Bedrijf Huisvesting
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:30-01-1995

Name	:Peters, Johannes Reinier Theodorus Maria
Date and place of birth	:01-10-1940, Nijmegen
Address	:Dorpenweg 25, 5371KS Ravenstein
Date of entry into office	:01-06-1992
Title	:Lid college van bestuur
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-06-1992

Name	:Veenstra, Wopke Mient
Date and place of birth	:17-08-1951, Den Ham
Address	:Jonckherenhof 2, 6581GD Malden
Date of entry into office	:01-01-1995
Title	:Directeur uci
Powers	:Restricted power of attorney

20,00 04-05-2004 Page 00005 follows.

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Commencement (present) power of attorney	:01-07-1998
Name	:Simons, Peter Robert Jan
Date and place of birth	:15-05-1949, Batavia, Dutch East Indies
Address	:Gietersstraat 8, 1015HB Amsterdam
Date of entry into office	:01-06-1994
Title	:Directeur Nuovo
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-1998
Name	:Tonnaer, Leonardus Johannes Gertrude Joseph Marie Irène
Date and place of birth	:17-09-1944, Amby
Address	:Rijksweg 124, 6585AJ Mook
Date of entry into office	:01-01-1995
Title	:Directeur arbo-en milieudienst
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-1998
Name	:Husken, Franciscus Adelbertus Maria
Date and place of birth	:28-09-1945, Nijmegen
Address	:Zijlweg 289, 2015CM Haarlem
Date of entry into office	:01-08-1995
Title	:Directeur NOM
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-1998
Name	:van den Boogaard, Petrus Hendricus Wilhelmus
Date and place of birth	:14-03-1952, Nijmegen
Address	:Onyxstraat 12, 6534XX Nijmegen
Date of entry into office	:01-06-1996
Title	:Hoofd fa-debiteurenadministratie
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-12-2000
Name	:Coenen, Antonius Matheus Louis
Date and place of birth	:24-01-1943, Maasbree
Address	:Eikenlaan 33, 6584BT Molenhoek Lb

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Date of entry into office	:01-05-1997
Title	:Directeur Onderw. Inst. P&C
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-1998
Name	:Meijer, Gerardus Jacobus Wilhelmus
Date and place of birth	:01-11-1966, Oeffelt
Address	:Veldzuring 12, 5432GP Cuijk
Date of entry into office	:01-03-1998
Title	:UMC: strategisch inkoper
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-03-1998
Name	:Dankbaar, Bernard
Date and place of birth	:29-07-1948, Amsterdam
Address	:Straalmanstraat 7, 6521JK Nijmegen
Date of entry into office	:01-09-1999
Title	:Decaan fac. managementwetenschappen
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-09-1999
Name	:Wilke, Ronald Justinus
Date and place of birth	:14-04-1959, Leidschendam
Address	:Herman Oolbekkinkstraat 8, 6523RB Nijmegen
Date of entry into office	:01-04-2001
Title	:Directeur UFB
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-04-2001
Name	:Schmitz, Jan Jaap Aloysius
Date and place of birth	:21-06-1954, 's-Gravenhage
Address	:Jonkershof 10, 6561AL Groesbeek
Date of entry into office	:01-10-2001
Title	:UMC:directeur facilitair bedrijf
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-10-2001
Name	:Schalkwijk, Eus Valentijn

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Date and place of birth	:14-08-1960, Zutphen
Address	:Groenewoudseweg 375, 6525EL Nijmegen
Date of entry into office	:01-02-2002
Title	:Directeur IOWO
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-02-2002
Name	:Brand, Willem
Date and place of birth	:26-08-1952, Sneek
Address	:Kleidonk 11, 6641LM Beuningen Gld
Date of entry into office	:01-12-2001
Title	:Directeur dienst studentenzaken
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-12-2001
Name	:Cirkel, Guurtje Hermina
Date and place of birth	:29-07-1952, Amersfoort
Address	:Groesbeekseweg 172, 6521CR Nijmegen
Date of entry into office	:01-04-2002
Title	:Directeur URD
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-04-2002
Name	:Frik, Adrianus Johannes
Date and place of birth	:06-02-1943, Hilvarenbeek
Address	:Rembrandtstraat 77, 6521MD Nijmegen
Date of entry into office	:01-02-2002
Title	:Directeur lerarenopleiding KUN/ILS
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2002
Name	:van Zoelen, Everardus Johannes Jacobus
Date and place of birth	:27-02-1951, Rotterdam
Address	:Fangmanweg 43, 6862EH Oosterbeek
Date of entry into office	:01-11-2001
Powers	:Full power of attorney
Commencement (present) power of attorney	:01-11-2001

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KAMER VAN KOOPHANDEL
CENTRAAL GELDERLAND

File number: 41055629 Page 00008

Name	:Brand, Willem
Date and place of birth	:26-08-1952, Sneek
Address	:Kleidonk 11, 6641LM Beuningen Gld
Date of entry into office	:01-01-2003
Title	:Dir. dienst studentenzaken
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:Lippmann-Duijts, Josepha Antonia Maria
Date and place of birth	:15-01-1946, Arnhem
Address	:Meppelstraat 22, 6835HD Arnhem
Date of entry into office	:01-07-2002
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-2002
Name	:van Borssum Buisman, Warmold Hendrik
Date and place of birth	:02-01-1952, Wassenaar
Address	:Imbosch 2 -3, 6961LJ Eerbeek
Date of entry into office	:01-05-2003
Title	:Ad intrim Cluster Facilitair
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-05-2003
Name	:van der Kroft, Johanna Catharina Maria
Date and place of birth	:19-05-1952, Heerlen
Address	:Evertsenlaan 24, 6881GC Velp Gld
Date of entry into office	:01-01-2003
Title	:Dir. diensten personeel & organisatie
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:Jansen, Cornelis Johannes Henricus
Date and place of birth	:01-09-1961, Geldrop
Address	:Albert Schweitzerlaan 96, 6525JV Nijmegen
Date of entry into office	:01-05-2003
Title	:Beheerder fac. rechtsgeleerdheid
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-05-2003

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Name	:Cuppen, Robertus Maria Antonius
Date and place of birth	:18-12-1954, Breda
Address	:Eikendreef 67, 6581PC Malden
Date of entry into office	:01-01-2003
Title	:Dir. universitair sportcentrum
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:Janssen, Maria Lina Margaretha
Date and place of birth	:23-10-1953, Deventer
Address	:Charles Estourgiestraat 6, 6522HW Nijmegen
Date of entry into office	:01-01-2003
Title	:Hoofd externe relaties
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:van de Woestijne, Johannes Christiaan
Date and place of birth	:13-02-1951, Maarn
Address	:Kruisstraat 9, 6576JR Ooij
Date of entry into office	:01-01-2003
Title	:Hoofd communicatie
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:van Pelt, Adrianus Christianus Andreas
Date and place of birth	:31-01-1946, Teteringen
Address	:Tolschestraat 45, 5363TB Velp NB
Date of entry into office	:01-07-2002
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-2002
Name	:Nissen, Petrus Johannes Andreas
Date and place of birth	:09-10-1957, Swalmen
Address	:Berg en Dalseweg 296, 6522CN Nijmegen
Date of entry into office	:01-05-2003
Title	:Beheerder fac. der theologie
Powers	:Restricted power of attorney
Commencement (present)	

20,00 04-05-2004

Page 00010 follows.

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KAMER VAN KOOPHANDEL
CENTRAAL GELDERLAND

File number: 41055629 Page 00010

power of attorney	:01-05-2003
Name	:Maalderink, Henricus Gerardus
Date and place of birth	:29-01-1975, Warnsveld
Address	:Vergert 29, 6662DX Elst Gld
Date of entry into office	:01-08-2003
Title	:Hoofd Financieel Economisch Beleid & Control
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-08-2003
Name	:Winkels, Jeroen Wilhelmus
Date and place of birth	:26-07-1956, Groningen
Address	:Hondiusdomein 8, 6229GH Maastricht
Date of entry into office	:01-04-2002
Title	:Directeur ITS
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-04-2002

Issued by the chamber of commerce

184,00 Arnhem, 04-05-2004

KAMER VAN KOOPHANDEL CENTRAAL GELDERLAND

M.Th H. Fontein-Duynhoven

HOOFDKANTOOR
KRONENBURGSINGEL 525
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SEPTEMBER 01, 2004

PTAS

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C
1100 NEW YORK AVENUE, N.W.
WASHINGTON, DC 20005

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RECORDATION DATE: 08/31/2004 REEL/FRAME: 015060/0902
NUMBER OF PAGES: 14

BRIEF: CORRECTIVE ASSIGNMENT TO CORRECT THE SPELLING OF THE CITY IN THE RECEIVING PARTY SECTION PREVIOUSLY RECORDED ON REEL 015020 FRAME 0591. ASSIGNOR(S) HEREBY CONFIRMS THE SPELLING OF THE CITY IN THE RECEIVING PARTY SECTION SHOULD BE \"NIJMEGEN\" NOT \"NIJMEGAN\".

ASSIGNOR:
UNIVERSITY HOSPITAL NIJMEGEN DOC DATE: 11/01/1999

ASSIGNEE:
UNIVERSITY MEDICAL CENTRE NIJMEGEN
GEERT GROOTEPLEIN 10
P.O. BOX 9101
NIJMEGEN, NETHERLANDS 6500 HB

SERIAL NUMBER: 09402713 FILING DATE: 06/13/2000
PATENT NUMBER: ISSUE DATE:
TITLE: PCA3, PCA3 GENES, AND METHODS OF USE

015060/0902 PAGE 2

DOROTHY RILEY, PARALEGAL
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

MARCH 27, 2006

PTAS

STERNE, KESSLER, GOLDSTEIN
& FOX P.L.L.C.
C/O CYNTHIA BOUCHEZ
1100 NEW YORK AVENUE, N.W.
WASHINGTON, D.C. 20005-3934

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PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 571-272-3350. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, MAIL STOP: ASSIGNMENT SERVICES BRANCH, P.O. BOX 1450, ALEXANDRIA, VA 22313.

RECORDATION DATE: 01/06/2006

REEL/FRAME: 017365/0965
NUMBER OF PAGES: 16

BRIEF: CORRECTIVE ASSIGNMENT TO CORRECT THE NAME OF THE ASSIGNEE PREVIOUSLY RECORDED ON REEL 015060, FRAME 0902. ASSIGNOR HEREBY CONFIRMS THE NAME OF THE ASSIGNEE SHOULD BE "STICHTING KATHOLIEKE UNIVERSITEIT, MORE PARTICULARLY THE UNIVERSITY MEDICAL CENTRE NIJMEGAN."

ASSIGNOR:
UNIVERSITY MEDICAL CENTRE NIJMEGEN DOC DATE: 11/01/1999

ASSIGNEE:
STICHTING KATHOLIEKE UNIVERSITEIT,
MORE PARTICULARLY THE UNIVERSITY
MEDICAL CENTRE NIJMEGEN
GEERT GROOTEPLEIN 10
P.O. BOX 9109
NIJMEGEN, NETHERLANDS 6500 HB

SERIAL NUMBER: 09402713 FILING DATE: 06/13/2000
PATENT NUMBER: 7008765 ISSUE DATE: 03/07/2006
TITLE: PCA3, PCA3 GENES, AND METHODS OF USE

MARY BENTON, EXAMINER
ASSIGNMENT SERVICES BRANCH
PUBLIC RECORDS DIVISION

UMC St Radboud

STATEMENT

Herewith we declare that:

- d.d. November 1st, 1999, the **Faculty of Medicine of the University of Nijmegen** integrated with the **University Hospital Nijmegen**;
- d.d. November 1st, 1999, these two entities together became one entity named the **University Medical Centre Nijmegen**, which is part of **Stichting Katholieke Universiteit**, a legal entity (foundation) by virtue of the Dutch 'Wet op het hoger onderwijs en wetenschappelijk onderzoek' (Higher Education and Academic Research Act) according to Dutch law.

Stichting Katholieke Universiteit,
more particularly the University Medical Centre Nijmegen

IN WITNESS WHEREOF, the ~~University Medical Centre Nijmegen~~ hereto has executed this Statement by her duly authorized representatives,

Nijmegen, 26.05.2004

Prof.dr. C.L.A. van Herwaarden
Chairman of the Executive Board

Nijmegen, 26.05.2004

mr. E.A.M. Kerckhoffs
Secretary of the Executive Board

KAMER VAN KOOPHANDEL
CENTRAAL GELDERLAND

File number: 41055629 Page 00001

English translation of an extract from the commercial register of the Chamber of Commerce and Industries for Centraal Gelderland

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Legal person:

Legal form	:Foundation
Corporate name	:Stichting Katholieke Universiteit
Abbreviated name	:Stichting K.U.
Statutory seat	:Nijmegen
Address	:Geert Grooteplein-Noord 9, 6525EZ Nijmegen
Mailing address	:Postbus 9102, 6500HC Nijmegen
Telephone numbers	:024-3611555 / 024-3615444
Fax number	:024-3541468
Incorporation deed	:30-07-1905
Deed of latest amendment of articles	:27-10-1997
Business activities suspended as at	:01-03-2004

Director(s):

Name	:Grobbeé, Johannes Jacobus Jozef
Date and place of birth	:19-03-1940, Dalfsen
Address	:Pothoofd 116, 7411ZD Deventer
Date of entry into office	:27-03-1995
Powers	:Authorised jointly (with other directors, see articles)
Date of (present) representative authority	:27-03-1995

Name	:Lockefeer, Henricus Antonius Ludovicus
Date and place of birth	:23-10-1938, Roosendaal en Nispen
Address	:Jacob Obrechtlaan 13, 1401CE Bussum
Date of entry into office	:05-04-1995
Title	:Vice-voorzitter
Powers	:Solely/independently authorised
Date of (present) representative authority	:20-12-2002

Name	:Brentjens, Josephus Lambertus
Date and place of birth	:30-03-1940, Haelen
Address	:Koepellaan 6, 2061CV Bloemendaal
Date of entry into office	:11-03-1993
Title	:Voorzitter

24,00 04-05-2004 Page 00002 follows.

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KAMER VAN KOOPHANDEL
CENTRAAL GELDERLAND

File number: 41055629 Page 00002

Powers	:Solely/independently authorised
Date of (present) representative authority	:19-10-2001
Name	:Splinter-van Kan, Hubertina Anna Gertruda
Date and place of birth	:09-06-1947, Maastricht
Address	:Prinsengracht 698, 1017LA Amsterdam
Date of entry into office	:11-03-1993
Powers	:Authorised jointly (with other directors, see articles)
Date of (present) representative authority	:11-03-1993
Name	:de Wijkerslooth de Weerdesteijn, Roelof Jozef
Date and place of birth	:18-10-1946, 's-Gravenhage
Address	:Theresiaweg 2, 6523ND Nijmegen
Date of entry into office	:01-05-2000
Title	:Voorzitter van het college van bestuur
Powers	:Solely/independently authorised
Date of (present) representative authority	:01-05-2000
Name	:Vugts, Johannes Franciscus Theresia
Date and place of birth	:10-07-1942, 's-Gravenhage
Address	:Bergweg 24, 6523MD Nijmegen
Date of entry into office	:01-01-2003
Powers	:Authorised jointly (with other directors, see articles)
Date of (present) representative authority	:01-01-2003
Name	:Wiggers-Rust, Liduina Francisca
Date and place of birth	:12-10-1951, Nijmegen
Address	:Burg.de Millylaan 8, 7231DR Warnsveld
Date of entry into office	:01-09-2002
Powers	:Authorised jointly (with other directors, see articles)
Date of (present) representative authority	:01-09-2002
Name	:van Herwaarden, Cornelis Lambertus August
Date and place of birth	:25-01-1944, Nijmegen
Address	:Kwakkenbergweg 57, 6523ML Nijmegen

20,00 04-05-2004 Page 00003 follows.

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Date of entry into office	:01-12-2003
Title	:UMC: voorzitter raad van bestuur
Powers	:Solely/independently authorised
Date of (present) representative authority	:01-12-2003
Name	:Ruiter, Dirk Jacob
Date and place of birth	:26-06-1947, Harlingen
Address	:Vinkenlaan 14, 6581CK Malden
Date of entry into office	:01-01-2004
Title	:UMC: vice voorzitter raad van bestuur/ decaan
Powers	:Solely/independently authorised
Date of (present) representative authority	:01-01-2004

Authorized signatory(signatories):

Name	:Hegeman, Wilhelmus Everhardus Joseph
Date of birth	:20-03-1948
Address	:Violenstraat 32, 6602CL Wijchen
Date of entry into office	:30-01-1995
Title	:UMC: strategisch inkoper
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:30-01-1995
Name	:de Bekker, Michaël Johannes Jozef
Date and place of birth	:21-07-1957, Lith
Address	:Deken Fritsenstraat 71, 5243VM Rosmalen
Date of entry into office	:01-05-1992
Title	:UMC: Directeur financieel economische zaken
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-05-1992
Name	:Hesseling, Jozef Hendrik
Date and place of birth	:14-02-1946, Arnhem
Address	:Zevendreef 3034, 6605VC Wijchen
Date of entry into office	:01-11-1991
Title	:UMC: strategisch inkoper
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-11-1991

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Name	:Kerckhoffs, Aemilius Antonius Maria
Date and place of birth	:11-10-1949, Nijmegen
Address	:Jaspisstraat 3, 6534SE Nijmegen
Date of entry into office	:01-11-1991
Title	:UMC: secretaris Raad van Bestuur
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-11-1991

Name	:Schils, Denis Antoon Gerard Jérôme
Date and place of birth	:15-10-1940, Roosendaal en Nispen
Address	:Gildestraat 39, 6691DT Gendt
Date of entry into office	:01-10-1993
Title	:Secretaris stg kun
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-10-1993

Name	:van Baal, Felix Marcus Jacobus Maria
Date and place of birth	:21-10-1957, Nijmegen
Address	:Witte Hoeflaan 24, 5343EH Oss
Date of entry into office	:30-01-1995
Title	:UMC: Algemeen directeur Bedrijf Huisvesting
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:30-01-1995

Name	:Peters, Johannes Reinier Theodorus Maria
Date and place of birth	:01-10-1940, Nijmegen
Address	:Dorpenweg 25, 5371KS Ravenstein
Date of entry into office	:01-06-1992
Title	:Lid college van bestuur
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-06-1992

Name	:Veenstra, Wopke Mient
Date and place of birth	:17-08-1951, Den Ham
Address	:Jonckherenhof 2, 6581GD Malden
Date of entry into office	:01-01-1995
Title	:Directeur uci
Powers	:Restricted power of attorney

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Commencement (present) power of attorney	:01-07-1998
Name	:Simons, Peter Robert Jan
Date and place of birth	:15-05-1949, Batavia, Dutch East Indies
Address	:Gietersstraat 8, 1015HB Amsterdam
Date of entry into office	:01-06-1994
Title	:Directeur Nuovo
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-1998
Name	:Tonnaer, Leonardus Johannes Gertrude Joseph Marie Irène
Date and place of birth	:17-09-1944, Amby
Address	:Rijksweg 124, 6585AJ Mook
Date of entry into office	:01-01-1995
Title	:Directeur arbo-en milieudienst
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-1998
Name	:Husken, Franciscus Adelbertus Maria
Date and place of birth	:28-09-1945, Nijmegen
Address	:Zijlweg 289, 2015CM Haarlem
Date of entry into office	:01-08-1995
Title	:Directeur NOM
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-1998
Name	:van den Boogaard, Petrus Hendricus Wilhelmus
Date and place of birth	:14-03-1952, Nijmegen
Address	:Onyxstraat 12, 6534XX Nijmegen
Date of entry into office	:01-06-1996
Title	:Hoofd fa-debiteurenadministratie
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-12-2000
Name	:Coenen, Antonius Matheus Louis
Date and place of birth	:24-01-1943, Maasbree
Address	:Eikenlaan 33, 6584BT Molenhoek Lb

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Date of entry into office	:01-05-1997
Title	:Directeur Onderw. Inst. P&C
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-1998
Name	:Meijer, Gerardus Jacobus Wilhelmus
Date and place of birth	:01-11-1966, Oeffelt
Address	:Veldzuring 12, 5432GP Cuijk
Date of entry into office	:01-03-1998
Title	:UMC: strategisch inkoper
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-03-1998
Name	:Dankbaar, Bernard
Date and place of birth	:29-07-1948, Amsterdam
Address	:Straalmanstraat 7, 6521JK Nijmegen
Date of entry into office	:01-09-1999
Title	:Decaan fac. managementwetenschappen
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-09-1999
Name	:Wilke, Ronald Justinus
Date and place of birth	:14-04-1959, Leidschendam
Address	:Herman Oolbekkinkstraat 8, 6523RB Nijmegen
Date of entry into office	:01-04-2001
Title	:Directeur UFB
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-04-2001
Name	:Schmitz, Jan Jaap Aloysius
Date and place of birth	:21-06-1954, 's-Gravenhage
Address	:Jonkershof 10, 6561AL Groesbeek
Date of entry into office	:01-10-2001
Title	:UMC:directeur facilitair bedrijf
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-10-2001
Name	:Schalkwijk, Eus Valentijn

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Date and place of birth	:14-08-1960, Zutphen
Address	:Groenewoudseweg 375, 6525EL Nijmegen
Date of entry into office	:01-02-2002
Title	:Directeur IOWO
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-02-2002
Name	:Brand, Willem
Date and place of birth	:26-08-1952, Sneek
Address	:Kleidonk 11, 6641LM Beuningen Gld
Date of entry into office	:01-12-2001
Title	:Directeur dienst studentenzaken
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-12-2001
Name	:Cirkel, Guurtje Hermina
Date and place of birth	:29-07-1952, Amersfoort
Address	:Groesbeekseweg 172, 6521CR Nijmegen
Date of entry into office	:01-04-2002
Title	:Directeur URD
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-04-2002
Name	:Frik, Adrianus Johannes
Date and place of birth	:06-02-1943, Hilvarenbeek
Address	:Rembrandtstraat 77, 6521MD Nijmegen
Date of entry into office	:01-02-2002
Title	:Directeur lerarenopleiding KUN/ILS
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2002
Name	:van Zoelen, Everardus Johannes Jacobus
Date and place of birth	:27-02-1951, Rotterdam
Address	:Fangmanweg 43, 6862EH Oosterbeek
Date of entry into office	:01-11-2001
Powers	:Full power of attorney
Commencement (present) power of attorney	:01-11-2001

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Name	:Brand, Willem
Date and place of birth	:26-08-1952, Sneek
Address	:Kleidonk 11, 6641LM Beuningen Gld
Date of entry into office	:01-01-2003
Title	:Dir. dienst studentenzaken
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:Lippmann-Duijts, Josepha Antonia Maria
Date and place of birth	:15-01-1946, Arnhem
Address	:Meppelstraat 22, 6835HD Arnhem
Date of entry into office	:01-07-2002
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-2002
Name	:van Borssum Buisman, Warmold Hendrik
Date and place of birth	:02-01-1952, Wassenaar
Address	:Imbosch 2 -3, 6961LJ Eerbeek
Date of entry into office	:01-05-2003
Title	:Ad intrim Cluster Facilitair
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-05-2003
Name	:van der Kroft, Johanna Catharina Maria
Date and place of birth	:19-05-1952, Heerlen
Address	:Evertsenlaan 24, 6881GC Velp Gld
Date of entry into office	:01-01-2003
Title	:Dir. diensten personeel & organisatie
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:Jansen, Cornelis Johannes Henricus
Date and place of birth	:01-09-1961, Geldrop
Address	:Albert Schweitzerlaan 96, 6525JV Nijmegen
Date of entry into office	:01-05-2003
Title	:Beheerder fac. rechtsgeleerdheid
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-05-2003

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Name	:Cuppen, Robertus Maria Antonius
Date and place of birth	:18-12-1954, Breda
Address	:Eikendreef 67, 6581PC Malden
Date of entry into office	:01-01-2003
Title	:Dir. universitair sportcentrum
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:Janssen, Maria Lina Margaretha
Date and place of birth	:23-10-1953, Deventer
Address	:Charles Estourgiestraat 6, 6522HW Nijmegen
Date of entry into office	:01-01-2003
Title	:Hoofd externe relaties
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:van de Woestijne, Johannes Christiaan
Date and place of birth	:13-02-1951, Maarn
Address	:Kruisstraat 9, 6576JR Ooij
Date of entry into office	:01-01-2003
Title	:Hoofd communicatie
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-01-2003
Name	:van Pelt, Adrianus Christianus Andreas
Date and place of birth	:31-01-1946, Teteringen
Address	:Tolschestraat 45, 5363TB Velp NB
Date of entry into office	:01-07-2002
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-07-2002
Name	:Nissen, Petrus Johannes Andreas
Date and place of birth	:09-10-1957, Swalmen
Address	:Berg en Dalseweg 296, 6522CN Nijmegen
Date of entry into office	:01-05-2003
Title	:Beheerder fac. der theologie
Powers	:Restricted power of attorney
Commencement (present)	

HOOFDKANTOOR
KRONENBURGSINGEL 525
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KAMER VAN KOOPHANDEL CENTRAAL GELDERLAND

File number: 41055629 Page 00010

power of attorney	:01-05-2003
Name	:Maalderink, Henricus Gerardus
Date and place of birth	:29-01-1975, Warnsveld
Address	:Vergert 29, 6662DX Elst Gld
Date of entry into office	:01-08-2003
Title	:Hoofd Financieel Economisch Beleid & Control
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-08-2003
Name	:Winkels, Jeroen Wilhelmus
Date and place of birth	:26-07-1956, Groningen
Address	:Hondiusdomein 8, 6229GH Maastricht
Date of entry into office	:01-04-2002
Title	:Directeur ITS
Powers	:Restricted power of attorney
Commencement (present) power of attorney	:01-04-2002

Issued by the chamber of commerce

184,00

Arnhem, 04-05-2004

KAMER VAN KOOPHANDEL CENTRAAL GELDERLAND

M.Th H. Fontein-Duynhoven

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JUN 14 2005

STERNE, KESSLER

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

JAG CMB Exhibit 8 MB 6/17/05

JUNE 08, 2005

PTAS

102914449A

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C
JORGE A. GOLDSTEIN
1100 NEW YORK AVENUE, N.W.
WASHINGTON, D.C. 20005-3934

UNITED STATES PATENT AND TRADEMARK OFFICE
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RECORDATION DATE: 12/23/2004

REEL/FRAME: 016106/0901
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
ISAACS, WILLIAM B.

DOC DATE: 12/21/2004

ASSIGNEE:
JOHNS HOPKINS UNIVERSITY, THE
3400 N. CHARLES STREET
BALTIMORE, MARYLAND 21218

SERIAL NUMBER: 09402713

FILING DATE: 06/13/2000

PATENT NUMBER:

ISSUE DATE:

TITLE: PCA3, PCA3 GENES, AND METHODS OF USE

PAULA MCCRAY, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

ASSIGNMENT

In consideration of the sum of One Dollar ($1.00) or equivalent and other good and valuable consideration paid to each of the undersigned inventor: **William B. Isaacs**, hereby sells and assigns to **The Johns Hopkins University**, whose mailing address is **3400 N. Charles Street, Baltimore, Maryland 21218** (hereafter referred to as the Assignee), his/her entire right, title and interest, including the right to sue for past infringement and to collect for all past, present and future damages, for the United States of America (as defined in 35 U.S.C. § 100) and throughout the world,

(a) in the invention(s) known as **PCA3, PCA3 Genes, and Methods of Use** for which application(s) for patent in the United States of America was filed on **June 13, 2000** (also known as United States Application No. **09/402,713**), in any and all applications thereon, in any and all Letters Patent(s) therefor, and

(b) in any and all applications that claim the benefit of the patent application listed above in part (a), including non-provisional applications, continuing (continuation, divisional, or continuation-in-part) applications, reissues, extensions, renewals and reexaminations of the patent application or Letters Patent therefor listed above in part (a), to the full extent of the term or terms for which Letters Patents issue, and

(c) in any and all inventions described in the patent application listed above in part (a), and in any and all forms of intellectual and industrial property protection derivable from such patent application, and that are derivable from any and all continuing applications, reissues, extensions, renewals and reexaminations of such patent application, including, without limitation, patents, applications, utility models, inventor's certificates, and designs together with the right to file applications therefor; and including the right to claim the same priority rights from any previously filed applications under the International Agreement for the Protection of Industrial Property, or any other international agreement, or the domestic laws of the country in which any such application is filed, as may be applicable;

all such rights, title and interest to be held and enjoyed by the above-named Assignee, its successors, legal representatives and assigns to the same extent as all such rights, title and interest would have been held and enjoyed by the Assignor had this assignment and sale not been made.

The undersigned inventors agree to execute all papers necessary in connection with the application(s) and any non-provisional, continuing (continuation, divisional, or continuation-in-part), reissue, reexamination or corresponding application(s) thereof and also to execute separate assignments in connection with such application(s) as the Assignee may deem necessary or expedient.

The undersigned inventors agree to execute all papers necessary in connection with any interference or patent enforcement action (judicial or otherwise) related to the application(s) or any non-provisional, continuing (continuation, divisional, or continuation-in-part), reissue or reexamination application(s) thereof and to cooperate with the Assignee in every way possible in obtaining evidence and going forward with such interference or patent enforcement action.

The undersigned inventors hereby represent that he/she has full right to convey the entire interest herein assigned, and that he/she has not executed, and will not execute, any agreement in conflict therewith.

The undersigned inventors hereby grant Robert Greene Sterne, Esq., Registration No. 28,912; Edward J. Kessler, Esq., Registration No. 25,688; Jorge A. Goldstein, Esq., Registration No. 29,021; David K.S. Cornwell, Esq., Registration No. 31,944; Robert W. Esmond, Esq., Registration No. 32,893; Tracy-Gene G. Durkin, Esq., Registration No. 32,831; Michele A. Cimbala, Esq., Registration No. 33,851; Michael B. Ray, Esq., Registration No. 33,997; Robert E. Sokohl, Esq., Registration No. 36,013; Eric K. Steffe, Esq., Registration No. 36,688; Michael Q. Lee, Esq., Registration No. 35,239; Steven R. Ludwig, Esq., Registration No. 36,203; John M. Covert, Esq., Registration No. 38,759; Linda E. Alcorn, Esq., Registration No. 39,588; Lawrence B. Bugaisky, Esq., Registration No. 35,086; Donald J. Featherstone, Esq., Registration No. 33,876; Robert C. Millonig, Esq., Registration No. 34,395; Michael V. Messinger, Esq., Registration No. 37,575; Judith U. Kim, Esq., Registration No. 40,679; Timothy J. Shea, Jr., Esq., Registration No. 41,306; Patrick E. Garrett, Esq., Registration No. 39,987; all of STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C., 1100 New York Avenue, N.W., Washington, D.C. 20005-3934, power to insert in this assignment any further identification that may be necessary or desirable in order to comply with the rules of the United States Patent and Trademark Office for recordation of this document.

IN WITNESS WHEREOF, executed by the undersigned inventors on the date opposite his/her name.

Date: 12/21/04 Signature of Inventor: William B. Isaacs

343517_1.DOC

GEN-PROBE

Exhibit 9

March 23, 2012

Mary C. Till
Senior Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner for Patent Examination Policy
United States Patent and Trademark Office
P.O. Box 1450, Alexandria,
VA 22313-1450

Re: Patent Term Extension Application for United States Patent No. 7,008,765 B1
SKGF Ref: 2218.034STR0

Dear Ms. Till:

On behalf of Gen-Probe Incorporated, Marketing Applicant for Premarket Approval Application ("PMA") P100033 for PROGENSA® PCA3 Assay, its predecessors and affiliates, I hereby authorize the patent owners of record, The Johns Hopkins University ("TJHU") and Stichting Katholieke Universiteit, more particularly the University Medical Centre Nijmegen ("SKU-UMCN"), in connection with their application for extension of U.S. Patent No. 7,008,765 B1 ("the '765 patent"), to rely upon the activities of Gen-Probe Incorporated, its predecessors and affiliates, undertaken in connection with seeking approval by the U.S. Food and Drug Administration of PMA P100033. Gen-Probe Incorporated has been a sub-licensee since 2003 of DiagnoCure Inc., which is an exclusive licensee of both TJHU and SKU-UMCN, under the '765 patent.

Very truly yours,

R. William Bowen, Jr.
Sr. Vice President and General Counsel
Gen-Probe Incorporated

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UMC St Radboud

Exhibit 10

Mar 26, 2012

Mary C. Till
Senior Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner for Patent Examination Policy
United States Patent and Trademark Office
P.O. Box 1450, Alexandria,
VA 22313-1450

Re: Patent Term Extension Application for United States Patent No. 7,008,765 B1
SKGF Ref: 2218.034STR0

Dear Ms. Till:

This is to advise you that, as an authorized representative of Stichting Katholieke Universiteit, more particularly the Radboud University Nijmegen Medical Centre ("SKU-UMCN"), an owner of United States Patent No. 7,008,765 B1 ("the '765 patent"), I hereby authorize Gen-Probe Incorporated of 10210 Genetic Center Drive, San Diego, CA ("Gen-Probe") to file and prosecute a patent term extension application pursuant to 35 U.S.C. § 156 regarding the '765 patent ("the PTE Application") on behalf of SKU-UMCN, pursuant to 37 C.F.R. § 1.730(c).

I understand that counsel at Sterne, Kessler, Goldstein & Fox PLLC, 1100 New York Avenue, NW, Washington, DC 20005 ("SKGF"), will file and prosecute the PTE Application as Gen-Probe's representative, pursuant to 37 C.F.R. § 1.730(c), and I hereby grant SKGF any authorizations from SKU-UMCN necessary for SKGF to act in this capacity.

Very truly yours,

Hendrik-Jan Vos, M.Sc., Director of Valorisation/Technology Transfer MBa
Authorized Representative of the Executive Board
Stichting Katholieke Universiteit, more particularly
the University Medical Centre Nijmegen

Exhibit 11

March 22, 2012

Mary C. Till
Senior Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner for Patent Examination Policy
United States Patent and Trademark Office
P.O. Box 1450, Alexandria,
VA 22313-1450

Re: Patent Term Extension Application for United States Patent No. 7,008,765 B1
SKGF Ref: 2218.034STR0

Dear Ms. Till:

This is to advise you that, as an authorized representative of The Johns Hopkins University, an owner of United States Patent No. 7,008,765 B1 ("the '765 patent"), I hereby authorize Gen-Probe Incorporated of 10210 Genetic Center Drive, San Diego, CA ("Gen-Probe") to file and prosecute a patent term extension application pursuant to 35 U.S.C. § 156 regarding the '765 patent ("the PTE Application") on behalf of The Johns Hopkins University, pursuant to 37 C.F.R. § 1.730(c).

I understand that counsel at Sterne, Kessler, Goldstein & Fox PLLC, 1100 New York Avenue, NW, Washington, DC 20005 ("SKGF"), will file and prosecute the PTE Application as Gen-Probe's representative, pursuant to 37 C.F.R. § 1.730(c), and I hereby grant SKGF any authorizations from The Johns Hopkins University necessary for SKGF to act in this capacity.

Very truly yours,

R. Keith Baker

Senior Director

Authorized Representative
The Johns Hopkins University

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GEN-PROBE®

PROGENSA® PCA3

PROGENSA® PCA3 Assay

Exhibit 12

For *in vitro* diagnostic use.

General Information

Intended Use

The PROGENSA PCA3 Assay is an *in vitro* nucleic acid amplification test. The assay measures the concentration of prostate cancer gene 3 (PCA3) and prostate-specific antigen (PSA) RNA molecules and calculates the ratio of PCA3 RNA molecules to PSA RNA molecules (PCA3 Score) in post-digital rectal exam (DRE) first catch male urine specimens. The PROGENSA PCA3 Assay is indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PROGENSA PCA3 Assay results.

A PCA3 Score <25 is associated with a decreased likelihood of a positive biopsy. Prostatic biopsy is required for diagnosis of cancer.

Warning:
The PROGENSA PCA3 Assay should not be used for men with atypical small acinar proliferation (ASAP) on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.

Warning: *The Clinical Study only included men who were recommended for repeat biopsy. Therefore, the performance of the PROGENSA PCA3 Assay has not been established in men for whom a repeat biopsy was not already recommended.*

Limitations

A. The PCA3 Score is intended to be used in conjunction with serum prostate-specific antigen (PSA) and other risk indicators to guide appropriate patient management in the "at risk" population of men who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended based on current standard of care.

B. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this insert may result in erroneous results. Test results may be affected by improper specimen collection, technical error, or specimen mix-up.

C. Performance of the PROGENSA PCA3 Assay has not been established in men who undergo repeat biopsy less than 3 months or more than 7 years after their most recent negative biopsy (refer to *Clinical Performance*, Table 9).

D. Each laboratory must independently validate an LIS transfer process.

E. Reliable results are dependent on adequate urine specimen collection. Because the transport system used for this assay does not permit microscopic assessment of urine specimen adequacy, training of clinicians in proper urine specimen collection techniques is necessary. See *Specimen Collection, Transport, and Storage* for instructions.

F. The effect of medications known to affect serum PSA levels such as finasteride (Proscar, Propecia), dutasteride (Avodart), and anti-androgen therapy (Lupron) on PROGENSA PCA3 Assay performance was not evaluated.

G. Certain therapeutic and diagnostic procedures such as prostatectomy, radiation, prostate biopsy, and others may affect the viability of prostatic tissue and subsequently impact the PCA3 Score. The effect of these procedures on assay performance has not yet been evaluated. Samples for PROGENSA PCA3 Assay testing should be collected when the clinician believes prostate tissue has recovered.

H. Results from the PROGENSA PCA3 Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician and relevant guidelines in the decision for repeat biopsy.

I. Information from percent free PSA tests was not used in establishing the performance characteristics of the PROGENSA PCA3 Assay.

Summary and Explanation of the Test

The use of the serum prostate-specific antigen (PSA) test for prostate cancer screening has resulted in the biopsy diagnosis of smaller, previously undetected tumors, thus creating a new diagnostic dilemma: Only a fraction of men with increased serum PSA levels have detectable prostate cancer. Men with at least one negative biopsy often have persistently increased serum PSA, due primarily to enlarged prostates and benign prostatic hyperplasia (BPH). Yet, a significant proportion of men with slightly increased serum PSA (2.5-4.0 µg/L) either have, or will develop, clinically significant prostate cancer (12).

PCA3 (also known as "PCA3^{DD3}" or "DD3^{PCA3}") is a non-coding prostate-specific RNA (1) that is highly over-expressed in prostate cancer cells, with a median 66-fold up-regulation compared to adjacent benign tissue (11). In contrast, PSA gene expression is similar in cancerous and benign prostate cells; PSA RNA levels may therefore be used to normalize for the amount of prostate-specific RNA in molecular test samples. The feasibility of quantitative PCA3-based molecular testing from urine sediments (11) and from whole urine (9) has been demonstrated. The clinical feasibility of a PCA3-based test from whole urine has been demonstrated in men undergoing repeat biopsy (10, 12).

The PROGENSA PCA3 Assay utilizes whole urine collected following a digital rectal examination (DRE) consisting of three strokes per lobe. The DRE releases prostate cells through the prostate duct system into the urinary tract, where they can be collected in the first catch urine. The urine is processed by addition of Urine Transport Medium (UTM), which lyses the cells and stabilizes the RNA. PCA3 and PSA RNAs are quantified, and the PCA3 Score is determined based on the ratio of PCA3/PSA RNA multiplied by 1000. In addition to normalizing PCA3 signal, measurement of PSA RNA also serves to confirm that the yield of prostate-specific RNA is sufficient to generate a valid result.

Principles of the Procedure

The PROGENSA PCA3 Assay is comprised of two quantitative nucleic acid amplification tests. The assay combines the technologies of target capture, Transcription Mediated Amplification (TMA), and Hybridization Protection Assay (HPA) to streamline urine specimen processing, amplify target RNA, and detect amplicon, respectively.

When the PROGENSA PCA3 Assay is performed in the laboratory, the target RNA molecules are isolated from the urine specimens by target capture. Oligonucleotides ("capture oligonucleotides") that are complementary to sequence specific regions of the targets are hybridized to the targets in the urine specimen. A separate capture oligonucleotide is used for each target. The hybridized target is then captured onto magnetic microparticles that are separated from the urine specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube. Magnetic separation and wash steps are performed with a target capture system.

Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, Moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase. A unique set of primers is used for each target. The reverse transcriptase is used to generate a deoxyribonucleic acid (DNA) copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection is achieved by HPA using single-stranded, chemiluminescent-labeled nucleic acid probes that are complementary to the amplicon. Separate probes are used for each target amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured in a luminometer and is reported as Relative Light Units (RLU).

PCA3 and PSA RNAs are quantified in separate tubes and the PCA3 Score is determined. Calibrators containing known amounts of PCA3 or PSA RNA transcripts are included in every assay run and used to generate a standard curve. PCA3 and PSA controls are also included to verify the accuracy of results interpolated from the standard curve.

Reagents and Materials Provided

Reagents and materials provided in the PROGENSA PCA3 Assay Kit for the PROGENSA PCA3 Assay are listed below. Reagent Identification Symbols are also listed next to the reagent name.

PROGENSA PCA3 Assay Kit, 2 x 100 Reactions (302355)

PROGENSA PCA3 100-Reaction Kit

PROGENSA PCA3 Refrigerated Box — Store at 2°C to 8°C upon receipt until the labeled expiration date

Symbol	Component	Quantity
A	**PCA3 Amplification Reagent** *Non-infectious nucleic acids dried in HEPES buffered solution containing <10% bulking agent.*	1 vial
E	**PCA3/PSA Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing <10% bulking agent.*	1 vial
P	**PCA3 Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing <5% bulking agent and <5% lithium lauryl sulfate.*	1 vial

PROGENSA PCA3 Room Temperature Box — Store at 15°C to 30°C upon receipt until the labeled expiration date

Symbol	Component	Quantity
AR	**PCA3 Amplification Reconstitution Solution** *Aqueous solution containing preservatives (<1% parabens).*	1 x 9.3 mL
ER	**PCA3/PSA Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant (10% Triton X-100) and 20% glycerol.*	1 x 3.3 mL
PR	**PCA3/PSA Probe Reconstitution Solution** *Succinate buffered solution containing <5% lithium lauryl sulfate.*	1 x 12.4 mL
S	**PCA3/PSA Selection Reagent** *Borate buffered solution containing surfactant (1% Triton X-100).*	1 x 31mL
TCR	**PCA3 Target Capture Reagent** *Non-infectious nucleic acid in HEPES buffered solution containing solid phase.*	1 x 22 mL
	**Sealing Cards**	1 package
	**Reconstitution Collars**	1 package

PROGENSA PCA3 Calibrator and Controls Kit — Store at 2°C to 8°C upon receipt until the labeled expiration date

Symbol	Component	Quantity
CAL	**PCA3 Calibrator 1** *Phosphate buffered solution containing <5% lithium lauryl sulfate.*	1 x 2.0 mL
CAL	**PCA3 Calibrators 2-5** *Non-infectious PCA3 nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.*	4 x 1.7 mL
PC	**PCA3 Positive Controls** *Non-infectious PCA3 nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.*	2 x 1.7 mL
	**PCA3 Concentration Information Sheet**	1 sheet

PROGENSA PSA 100-Reaction Kit

PROGENSA PSA Refrigerated Box — Store at 2°C to 8°C upon receipt until the labeled expiration date

Symbol	Component	Quantity
A	**PSA Amplification Reagent** *Non-infectious nucleic acids dried in HEPES buffered solution containing <10% bulking agent.*	1 vial
E	**PCA3/PSA Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing <10% bulking agent.*	1 vial
P	**PSA Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing <5% bulking agent and <5% lithium lauryl sulfate.*	1 vial

PROGENSA PSA Room Temperature Box — Store at 15°C to 30°C upon receipt until the labeled expiration date

Symbol	Component	Quantity
AR	**PSA Amplification Reconstitution Solution** *Aqueous solution containing preservatives (<1% parabens).*	1 x 9.3 mL
ER	**PCA3/PSA Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant (10% Triton X-100) and 20% glycerol.*	1 x 3.3 mL
PR	**PCA3/PSA Probe Reconstitution Solution** *Succinate buffered solution containing <5% lithium lauryl sulfate.*	1 x 12.4 mL
S	**PCA3/PSA Selection Reagent** *Borate buffered solution containing surfactant (1% Triton X-100).*	1 x 31 mL
TCR	**PSA Target Capture Reagent** *Non-infectious nucleic acid in HEPES buffered solution containing solid phase.*	1 x 22 mL
	**Sealing Cards**	1 package
	**Reconstitution Collars**	1 package

PROGENSA PSA Calibrator and Controls Kit — Store at 2°C to 8°C upon receipt until the labeled expiration date

Symbol	Component	Quantity
CAL	**PSA Calibrator 1** *Phosphate buffered solution containing <5% lithium lauryl sulfate.*	1 x 2.0 mL
CAL	**PSA Calibrators 2-5** *Non-infectious PSA nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.*	4 x 1.7 mL
PC	**PSA Positive Controls** *Non-infectious PSA nucleic acid in phosphate buffered solution containing <5% lithium lauryl sulfate.*	2 x 1.7 mL
	**PSA Concentration Information Sheet**	1 sheet

APTIMA Assay Fluids — Store at 15°C to 30°C (2 boxes) upon receipt until the labeled expiration date

Symbol	Component	Quantity
W	**Wash Solution** *HEPES buffered solution containing <2% sodium dodecyl sulfate.*	1 x 402 mL
DF	**Buffer for Deactivation Fluid** *Bicarbonate buffered solution.*	1 x 402 mL
O	**Oil Reagent** *Silicone oil.*	1 x 24.6 mL

Materials

Note: *Materials available from Gen-Probe have catalog numbers listed.*

Materials Required But Available Separately

	Cat. No.
PROGENSA PCA3 Urine Specimen Transport Kit	302352
GEN-PROBE LEADER HC+ Luminometer	104747
GEN-PROBE Target Capture System (TCS)	104555
APTIMA Auto Detect Kit	301048
2 eppendorf Repeater Plus Pipettors	105725
Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL)	—
2 to 4 SB100 Dry Heat Bath/Vortexers	105524
Micropipettor, 1000 µL RAININ PR1000	901715
Tips, 1000 µL P1000	105049
Bleach (minimum 5.25% or 0.7 M sodium hypochlorite solution)	—
Large-capped plastic container	—
Standard urine collection containers, without preservatives	—
Ten Tube Units (TTU)	TU0022
Ten Tip Cassettes (TTC)	104578
SysCheck calibration standard	301078

Optional Materials

		Cat. No.
PROGENSA PCA3 100-Reaction Kit		302354
PROGENSA PSA 100-Reaction Kit		302357
PROGENSA PCA3 Calibrators and Controls Kit		302353
PROGENSA PSA Calibrators and Controls Kit		302356
PROGENSA PCA3/PSA Proficiency Panels		302350
PROGENSA PCA3 Specimen Diluent Kit		302351
APTIMA Assay Fluids Kit		302002C
TECAN Freedom EVO 100/4 instrument		900932
PCA3 Deck Plate assembly, DTS 800	*902021*	
Reagent reservoir (40 mL quarter module)	*104765*	
Split reagent reservoir (19 mL x 2 quarter module)	*901172*	
Disposable pipet tips with filter (1 mL)	*10612513 (Tecan)*	

Transport tubes	302521
Pipettor, eppendorf 20 to 200 µL	105726
Tips, Pipette 20 to 200 µL	—
Replacement penetrable caps	302520
Replacement non-penetrable caps	103036A

Warnings and Precautions

A. For *in vitro* diagnostic use.

Laboratory Related

B. Use only supplied or specified disposable laboratory ware.

C. Use routine laboratory precautions. Do not eat, drink, or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling urine specimens and kit reagents. Wash hands thoroughly after handling urine specimens and kit reagents.

D. **Warning: Irritants, Corrosives.** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash with water. If these fluids spill, dilute the spill with water before wiping dry.

E. Work surfaces, pipettors, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution (see *Procedural Notes*).

F. A separate area for post-amplification is strongly recommended to minimize amplicon contamination in the assay. This dedicated area should be away from the pre-amplification area, where reagent preparation, target capture, and amplification take place.

G. To help prevent lab areas from becoming contaminated with amplicon, the laboratory area should be arranged with a unidirectional workflow from reagent preparation through post-amplification. Specimens, equipment, and reagents should not be returned to the area where a previous step was performed. Personnel should not move back into previous work areas without proper contamination safeguards. Only one run per shift should be performed.

Specimen Related

H. After urine has been added in the urine specimen transport tube, the liquid level must initially fall between the two black indicator lines on the tube label (at least 2.5 mL of urine is required). Otherwise, the specimen must be rejected.

I. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

J. Expiration dates listed on the collection kits pertain to the collection site and not the testing facility. Samples collected any time prior to the expiration date of the collection kit, and transported and stored in accordance with the package insert, are valid for testing even if the expiration date of the collection tube has passed.

K. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See *Specimen Collection, Transport, and Storage* for specific instructions.

L. Urine specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately qualified as proficient in the use of the PROGENSA PCA3 Assay and adequately trained in handling infectious materials should perform this procedure.

M. Avoid cross-contamination during the specimen handling steps. Urine specimens can contain high levels of RNA target. Ensure specimen containers do not contact one another, and discard used materials without passing them over any containers. If gloves come in contact with a specimen, change gloves to avoid cross-contamination.

Assay Related

N. Do not use this kit after its expiration date.

O. **For the PROGENSA PCA3 Assay kit, do not interchange, mix, or combine assay reagents with different lot numbers** (i.e., for each analyte, the assay reagents in the "refrigerated" box and "room temperature" box must come from the same lot).

P. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See *Storage and Handling Requirements* for specific instructions.

Q. For assay deactivation (see *DTS Systems Test Procedure*), the sodium hypochlorite concentration must be at least 2.6% (0.35 M) **after** 1:1 dilution with Buffer for Deactivation Fluid. Therefore, the starting concentration must be a minimum 5.25% (0.7 M) sodium hypochlorite to achieve the final concentration required for deactivation.

R. Tips with hydrophobic plugs must be used. A minimum of two repeat pipettors must be dedicated for use with this assay: one for use in the pre-amplification steps, and one for use in the post-amplification steps. One micropipettor must be dedicated for use in specimen transfer unless a TECAN Freedom EVO 100/4 instrument is used. All pipettors must be cleaned regularly as described in *Procedural Notes*.

S. When using repeat pipettors for reagent addition, do not touch the reaction tube with the pipettor tip to prevent carryover from one tube to another.

T. Separate SB100 instruments must be dedicated to the pre-amplification area for target capture and TMA and the post-amplification area for HPA.

U. Material Safety Data Sheets can be viewed online at www.gen-probe.com and are available upon request.

Storage and Handling Requirements

A. Consult Table 1 for reagent storage information.

Table 1: Reagent Storage

Reagent/Fluid	Unopened Storage	Opened/Reconstituted Stability (up to expiration date)
Amplification Reagents	2°C to 8°C until the expiration date	30 days at 2°C to 8°C*
Probe Reagents	2°C to 8°C until the expiration date	30 days at 2°C to 8°C*
Enzyme Reagent	2°C to 8°C until the expiration date	30 days at 2°C to 8°C*
Target Capture Reagents	15°C to 30°C until the expiration date	30 days at 15°C to 30°C
Amplification Reconstitution Solution	2°C to 30°C until the expiration date	N/A (single-use)
Probe Reconstitution Solution	2°C to 30°C until the expiration date	N/A (single-use)
Enzyme Reconstitution Solution	2°C to 30°C until the expiration date	N/A (single-use)
Selection Reagent	2°C to 30°C until the expiration date	30 days at 15°C to 30°C
Calibrators	2°C to 8°C until the expiration date	N/A (single-run)
Controls	2°C to 8°C until the expiration date	N/A (single-run)
Oil Reagent	15°C to 30°C until the expiration date	30 days at 15°C to 30°C
Wash Solution	15°C to 30°C until the expiration date	30 days at 15°C to 30°C
Buffer for Deactivation Fluid	15°C to 30°C until the expiration date	28 days at 15°C to 30°C

*May use again for other assay runs up to four times, provided that the total amount of time at room temperature is no greater than 24 hours.

B. **Do not store the Target Capture Reagent at temperatures below 15°C.**

C. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Protect these reagents from extended exposure to light during storage and preparation for use.

D. **Do not freeze the reagents.**

E. Do not use reagents or fluids after the expiration date.

F. PROGENSA PCA3 and PSA Calibrators and Controls are single-run vials and must be discarded after use.

G. Changes in the physical appearance of the reagent supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed once resuspended (e.g., obvious changes in reagent color or cloudiness indicative of microbial contamination), contact Gen-Probe Technical Support before use.

H. Discard reconstituted reagent after 30 days or by the expiration date, whichever comes first.

I. Leftover opened or reconstituted reagents may be used in subsequent assays if they have been stored properly after the initial use. The leftover reagent may be pooled with freshly prepared or other leftover reagent of the same lot. **Do not interchange, mix, or combine assay reagents with different lot numbers** (see *Warnings and*

Precautions). No components of the pooled reagent may exceed the opened or reconstituted reagent storage limits. Ensure that the pooled reagent has been thoroughly mixed and that sufficient volume has been prepared to provide enough reagent for an entire assay run.

Specimen Collection, Transport, and Storage

The PROGENSA PCA3 Assay is designed to quantify PCA3 and PSA RNA in first catch urine collected following a DRE consisting of three strokes per lobe. Urine is processed using the PROGENSA PCA3 Urine Specimen Transport Kit. Stability of PCA3 and PSA RNA in urine and processed urine was established by monitoring RNA copy levels in urine specimens collected per the instructions below.

A. Instructions for urine specimen collection and processing (for additional details, see "PROGENSA Physician Instructions" and "PROGENSA PCA3 Urine Specimen Transport Kit" instructions label):

1. Conduct a DRE as described below immediately prior to urine collection:

   Apply enough pressure to slightly depress the prostate surface, from the base to the apex and from the lateral to the median line for each lobe as shown in Figure 1. Perform exactly three strokes for each lobe. This is **NOT** intended to be a prostatic massage.

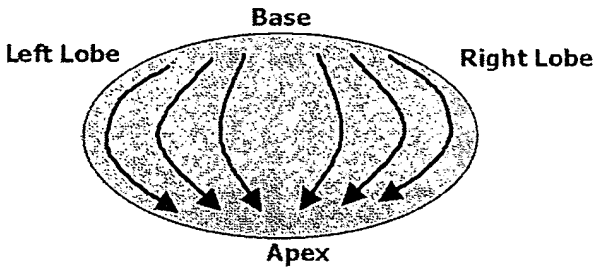


Figure 1. Proper Direction of Applied Prostate Pressure

2. Following the DRE, direct the patient to provide first catch urine (approximately 20 to 30 mL of the initial urine stream) in an appropriately labeled urine collection cup. This must be the first voided urine specimen following the DRE. Use a collection cup free of any preservatives. If a patient cannot stop his urine flow and provides more urine than the requested first 20 to 30 mL, keep the entire volume. Very high urine volumes can lower PCA3 and PSA analyte concentrations, and may infrequently result in an invalid specimen. Thus, the patient should try to avoid filling the urine collection cup. If the patient is unable to provide the requested volume of urine, at least 2.5 mL is required to run the PROGENSA PCA3 Assay. Otherwise, the specimen must be rejected.
3. **Unprocessed urine specimens, if not immediately processed, must be maintained at 2°C to 8°C or kept on ice. The chilled, unprocessed urine specimen must be transferred into the urine specimen transport tube within 4 hours of collection. Otherwise, the specimen must be rejected and the urologist must collect a new specimen. Do not freeze unprocessed urine specimens.**
4. To process urine specimens, tightly cap and invert the urine specimen 5 times to resuspend cells. Remove the cap of the urine specimen transport tube and transfer 2.5 mL of the collected urine into the tube using the disposable transfer pipette provided. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine specimen transport tube label.

5. Re-cap the urine specimen transport tube tightly and invert the urine specimen 5 times to mix. This is now known as the processed urine specimen.

B. Specimen transport and storage before testing (for details, see "PROGENSA Physician Instructions" and "PROGENSA PCA3 Urine Specimen Transport Kit" instructions label):

1. Processed urine specimens must be transported to the laboratory in the urine specimen transport tube. They may be shipped under ambient conditions (without temperature control) or frozen. Shipping arrangements must be made to ensure specimens are received by the testing site within 5 days of collection. Upon receipt of the shipment, the laboratory should verify the date of specimen collection on the tube. If specimens were shipped under ambient conditions and are received greater than 5 days after specimen collection, the specimen must be rejected and a request for a new specimen should be made. The laboratory may store specimens at 2°C to 8°C for up to 14 days before disposal is required. If longer time periods are needed, refer to Table 2 for the allowable storage times at different temperatures.

Table 2: Processed Urine Specimen Storage Durations

Storage Temperature	Time
Processed specimen storage and shipment:	Up to 5 days*
After receipt at testing site:	
2°C to 8°C	Up to 14 days
−35°C to −15°C	Up to 11 months**
At or below −65°C	Up to 36 months**

*Time allowed for shipment under ambient conditions or frozen.

**Time allowed after refrigerated storage.

2. Processed urine specimens may be subjected to up to 5 freeze–thaw cycles.

C. Specimen storage after testing

1. Specimens that have been assayed must be stored upright in a rack.
2. The urine specimen transport tubes, if not recapped with an intact cap, should be covered with a new, clean plastic or foil barrier.
3. If assayed specimens need to be frozen or shipped, remove the penetrable cap and place new, non-penetrable caps on the urine specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. **Avoid splashing and cross-contamination.**

Note: *Specimens must be shipped in accordance with applicable national and international transportation regulations.*

DTS Systems Test Procedure

A. Work Area Preparation

1. Prior to starting the assay, wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow sodium hypochlorite solution to contact surfaces and pipettors for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reaction will be performed with clean, plastic-backed absorbent laboratory bench covers.
2. Place a sufficient number of Ten Tip Cassettes into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with Wash Solution and the aspirator is connected to the vacuum pump. (Refer to the *Target Capture System Operator's Manual*.)

B. Reagent Reconstitution and Preparation

Reagent reconstitution should be performed prior to beginning specimen transfer.

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the Reconstitution Solutions to reach room temperature before use.

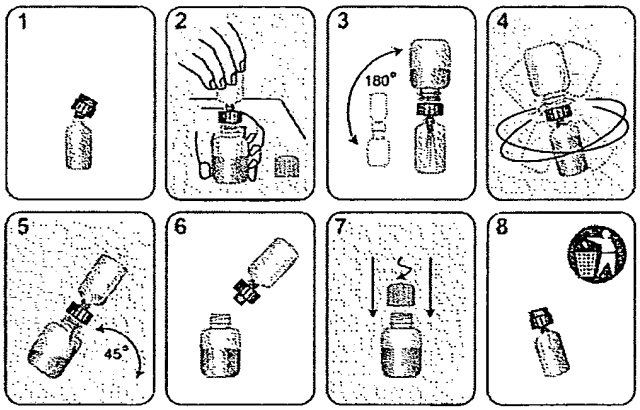


Figure 2. Reconstitution Process

a. Pair the appropriate reconstitution solution with its dried reagent. Verify that the vials have matching label colors to ensure they are paired properly.
b. Open the dried reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 2, Step 1).
c. Open the matching reconstitution solution, and set the cap on a clean, covered work surface. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 2, Step 2).
d. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 2, Step 3). Wait for the lyophilized reagent to go into solution, then gently swirl the solution in the glass vial to mix. Avoid creating foam while swirling the bottle (Figure 2, Step 4).
e. Invert the assembly and tilt at a 45° angle to minimize foaming (Figure 2, Step 5). Allow all of the liquid to drain back into the plastic bottle.

f. Remove the reconstitution collar and glass vial (Figure 2, Step 6).
g. Re-cap the plastic bottle (Figure 2, Step 7). Record operator initials and reconstitution date on all reconstituted reagent vials. Be sure to record the analyte (PCA3 or PSA) on the Probe Reagent vials.
h. Discard both the reconstitution collar and vial (Figure 2, Step 8).

2. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay. Refer to *Storage and Handling Requirements* if pooling leftover reagents. If reconstituted Amplification Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C ± 1°C for 1 to 2 minutes in the pre-amplification area. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C ± 1°C for 1 to 2 minutes in the post-amplification area. After these heat steps, the reconstituted reagents may be used even if residual precipitate remains. After resuspension, mix the vials by gentle inversion.

C. Rack Setup

The repeat pipettor used in target capture, specimen transfer and amplification should be dedicated for use in these steps only (see *Warnings and Precautions*).

1. Set up one rack for the PCA3 analyte and another rack for the PSA analyte.

   Note: *If the number of specimens is low enough, both analytes may be tested in a single rack. If using the TECAN Freedom EVO 100/4 instrument, separate racks must be maintained for each analyte. No more than two full racks (20 TTUs) may be tested at a time.*

2. In the Ten Tube Unit (TTU) rack(s), place enough TTUs to accommodate the calibrators, controls, and specimens for each analyte.
3. Label the TTUs with the sample/specimen IDs. Table 3 describes the addition of the calibrators, controls, and specimens. Start PSA calibrators on a new TTU.

   Note: *Calibrators are to be run in three replicates and controls in two replicates each, and must be run on the same rack as specimens. Specimens must be run in duplicate. Do not leave empty reaction tubes between calibrators, controls, and specimens. If using the TECAN Freedom EVO 100/4 instrument, refer to the TECAN Freedom EVO 100/4 Application Sheet for the PROGENSA PCA3 Assay (TECAN Freedom EVO Application Sheet) for further instructions.*

Table 3: Example Rack Setup

Rack Position	Sample Description	*Target PCA3 Concentration (copies/mL)	*Target PSA Concentration (copies/mL)
1 to 3	Calibrator 1	0	0
4 to 6	Calibrator 2	250	7,500
7 to 9	Calibrator 3	2,500	75,000
10 to 12	Calibrator 4	25,000	750,000
13 to 15	Calibrator 5	125,000	3,000,000
16 to 17	Control A	1,250	37,500
18 to 19	Control B	62,500	1,500,000
20 to n	Specimen	unknown	unknown

*PCA3 and PSA Positive Calibrators and Controls are value assigned, so the actual copies/mL values for Calibrators 2 to 5 and Controls A and B will be slightly different than the target concentrations listed in the table, and will vary from lot to lot. The concentration information is provided on a card in the package of calibrator and control vials and is used for calibration and determination of run validity.

D. Concentration Information Verification

Verify with the PROGENSA PCA3 Assay Software system administrator that the concentration information for the lots of PROGENSA PCA3 and PSA Calibrators and Controls Kits tested has been entered. For more information, see the *PROGENSA PCA3 Assay Software System Administrator's Manual*.

Note: *Entry of concentration information is required* ***before the first use*** *of each new calibrators and controls kit lot. Subsequent runs using calibrators and controls from the same kit lot do not require further action.*

E. Worklist Editor Setup

Generate an assay run worklist using the GEN-PROBE Worklist Editor on a computer located in the pre-amplification area. For use of the Worklist Editor, refer to the *GEN-PROBE Worklist Editor Operator's Manual*. If using the TECAN Freedom EVO 100/4 instrument, see also the *TECAN Freedom EVO Application Sheet* for further instructions.

F. Sample Preparation

1. Allow the calibrators and controls to reach room temperature prior to testing. Mix the vials by gentle inversion.
2. Allow specimens to reach room temperature prior to testing. **Do not vortex specimens.** The specimens should be mixed by occasional, gentle inversion during the warming period. See *Procedural Notes* for information about precipitate that will not go into solution and handling frozen specimens.

G. Pre-Amplification

The pre-amplification environment must be 15°C to 30°C. Run both racks in parallel. Refer to the *SB100 Dry Heat Bath/Vortexer Application Sheet* for the PROGENSA PCA3 Assay (*SB100 Application Sheet*) if necessary. If using the TECAN Freedom EVO 100/4 instrument, refer to the *TECAN Freedom EVO Application Sheet* for further instructions.

1. Prepare the pre-amp SB100 instrument for use.
   a. Press the "|" symbol on the power switch on the back of the pre-amp SB100 instrument(s). After successful initialization, the first screen displays the serial number and SB100 software/firmware version number.
   b. The **Main Menu** displays after 5 seconds:
   c. Press the ◀ key to select the **Select Run Mode** menu.
   d. Press the ▲ key to select the **Run Protocol** menu.
   e. Select the **PCA3 PREAMP V1.1** protocol on the pre-amp SB100 instrument by pressing the ▶ key and scrolling up or down through the name list using the ▲ or ▼ keys.
   f. Press the ↵ key to start the protocol. The SB100 instrument automatically preheats the sample block to 62°C.
2. Thoroughly mix by swirling or inverting the Target Capture Reagent (TCR). Using the repeat pipettor, add 100 µL of the analyte-specific TCR to the appropriate reaction tube.
3. Uncap the Calibrator vial or pierce the cap with the micropipettor and add 400 µL of the Calibrator to the properly labeled reaction tube. Use the same pipette tip to withdraw replicate additions from the vial. Use new pipette tips for each Calibrator vial. Repeat for the addition of Controls and specimens. Cover and save any leftover specimen and store at or below 8°C (see *Specimen Collection, Transport, and Storage* for more information) in case retesting is necessary.
4. Cover the TTUs with the sealing card(s). Cover the sealing card(s) with the SB100 frame.
5. When the sample block has reached 62°C, the SB100 instrument beeps. Load the rack in the SB100 sample block as indicated in the message on the screen. When finished, press the ▶ key to continue.

   Holding the frame and rack together, ease the rack into the sample block. Take care not to splash contents onto the sealing card(s). Rotate the black knobs until the bearings lock into the holes on the frame.

   Note: *When loading and unloading the rack in the sample block, hold the frame and rack assembly together to ensure TTUs are locked in position in the rack.*

   Note: *Ease the rack in or out of the sample block to avoid splashing contents on the sealing card(s). DO NOT JERK THE RACK.*

6. Press the ▶ key to continue. The SB100 instrument will:
   a. Vortex the rack for 10 seconds.
   b. Incubate the rack at 62°C for 35 minutes.
   c. Vortex the rack for 60 seconds.
   d. In the next 30 minutes, ramp down from 62°C to 23°C and incubate at 23°C until the end of the incubation.
7. When the SB100 instrument has completed the last incubation, the message on the screen indicates that target capture steps such as magnetic separation, aspiration, and wash will be performed next.
8. Gently remove the rack from the sample block. Take care not to splash contents onto the sealing card(s).
9. Place the rack with the Front tab forward on the TCS magnetic base for 5 to 10 minutes. Load the TTC rack with TTCs.
10. Prime the dispense station pump lines by pumping Wash Solution through the dispense manifold. Pump enough liquid through the system so that there are no air bubbles in the line and all ten nozzles are delivering a steady stream of liquid.
11. Turn on the vacuum pump and disconnect the aspiration manifold at the first connector between the aspiration manifold and the trap bottle. Ensure that the vacuum gauge meets the leak test specification. It may take 15 seconds to achieve this reading. Reconnect the manifold, and ensure that the vacuum gauge meets the vacuum level specification. Leave the vacuum pump on until all target capture steps are completed and the aspiration manifold tubing is dry.

    See the Target Capture System Vacuum Specifications Sheet located at the back of the *Target Capture System Operator's Manual* or contact Gen-Probe Technical Support for further information.
12. Firmly attach the aspiration manifold to the first set of tips. Lower the tips into the first TTU until the tips contact the top of the liquid. Maintain tip contact with the top of the liquid as the tips move downward until the tips come into brief contact with the bottoms of the tubes. Gently tap the tips against the bottoms of the tubes until all remaining liquid is removed. Do not hold the tips in prolonged contact with the bottoms of the tubes or tap the tips rapidly because excess foam may be created in the vacuum trap.
13. After the aspiration is complete, eject the tips into their original tip cassette. Repeat the aspiration steps for the remaining TTUs, using a dedicated tip for each reaction tube.
14. Place the dispense manifold over each TTU and, using the dispense station pump, deliver 1.0 mL of Wash Solution into each tube of the TTU.
15. Cover tubes with the sealing card(s) and remove the rack from the TCS.
16. Press the ▶ key to continue. Attach the frame, load the rack in the sample block, and lock the knobs into place over the frame.
17. Press the ▶ key to continue. The SB100 instrument will vortex the rack for 10 seconds.

18. After vortexing, the message on the screen indicates that target capture steps will be performed next.

    Remove the rack, and then press the ▶ key. The SB100 instrument will preheat the sample block to 62°C and will beep when 62°C is reached.

19. Place rack on the TCS magnetic base for 5 to 10 minutes.
20. The next SB100 message indicates the next steps are to aspirate Wash Solution, and then to add Amplification Reagent and Oil Reagent.
21. Aspirate all liquid as in Step 12 and Step 13.
22. After the final aspiration, remove the rack from the TCS base and visually inspect the tubes to ensure that all liquid has been aspirated, and all tubes contain magnetic particle pellets. If any liquid is visible, place the rack back onto the TCS base for 2 minutes, and repeat the aspiration for that TTU using the same tips used previously for each reaction tube. If ANY magnetic particle pellet is visible after aspiration is completed, the tube may be accepted. If no pellet is visible, the specimen should be retested. If the same specimen does not contain a magnetic particle pellet at this step in a subsequent run, this may indicate a specimen-specific problem. Re-collection of the urine specimen is recommended in this situation.

H. Amplification

Perform Step 5 on one rack before repeating on the second rack. If necessary, refer to the *SB100 Application Sheet*. If using the TECAN Freedom EVO 100/4 instrument, refer to the *TECAN Freedom EVO Application Sheet* for further instructions.

1. Using the repeat pipettor, add 75 µL of the reconstituted analyte-specific Amplification Reagent to each reaction tube. All reaction mixtures in the rack should be red in color.
2. Using the repeat pipettor, add 200 µL of Oil Reagent. Cover the tubes with the sealing card(s).
3. Press the ▶ key to continue. Load the rack in the SB100 sample block as indicated on the screen.

   Attach the frame, load the rack in the sample block, and then lock the knobs over the frame.

4. Press the ▶ key to continue. The SB100 instrument will:
   a. Vortex the rack for 10 seconds.
   b. Incubate the rack at 62°C for 10 minutes.
   c. Ramp down from 62°C to 42°C and incubate at 42°C until the end of the 5 minute incubation.

5. After the sample block has reached 42°C, the SB100 instrument will beep. The message on the screen indicates that Enzyme Reagent should be added.

   Remove the frame and discard the sealing card(s). Using the repeat pipettor, add 25 µL reconstituted Enzyme Reagent to each tube while the rack is in the sample block at 42°C. Immediately cover the tubes with fresh sealing card(s), attach the frame, and lock the knobs into place. Press the ▶ key.

   Note: The addition of enzyme must be completed within 90 seconds or less. *Complete this step on one rack before repeating on the second rack.*

   The SB100 instrument will:

   a. Gently vortex the rack for 5 seconds.
   b. Incubate the rack at 42°C for 60 minutes.

6. When the Enzyme incubation is complete, the message on the screen indicates that the rack should be removed for HPA.
7. Remove the rack and remove the frame, but leave the sealing card(s) on the tubes. The message on the screen indicates that the Pre-Amp protocol is complete.

   Note: *The rack must be removed within 5 minutes of completion of amplification. Otherwise, a Suspend Time error will occur. The error message indicates how much time has elapsed since it has been in error mode. Either continue or abort the protocol.*

8. Press any key to return to the **Main Menu**.
9. Turn off the pre-amp SB100 instrument(s) if there are no further tests to run. Proceed to the post-amplification area with the racks, covered with sealing card(s).

I. Post-Amplification

The repeat pipettor used in hybridization and selection should be dedicated for these steps only (see *Warnings and Precautions*). The post-amplification environment, including detection, must be 15°C to 30°C. If necessary, refer to the *SB100 Application Sheet.*

1. Prepare the post-amp SB100 instrument for use.
   a. Power up the post-amp SB100 instrument(s). The **Main Menu** displays after 5 seconds.
   b. Press the ◀ key to select the **Select Run Mode** menu.
   c. Press the ▲ key to select the **Run Protocol** menu.
   d. Select the **PCA3 PSTAMP V1.1** protocol on the post-amp SB100 instrument by pressing the ▶ key and scrolling up or down through the name list using the ▲ or ▼ keys.
   e. Press the ↵ key to start the protocol. The SB100 instrument will preheat the sample block to 62°C.

2. Hybridization
   a. When the sample block has reached 62°C, the SB100 instrument will beep. The message on the screen indicates to add Probe and load the rack in the SB100 sample block. Remove the sealing card(s), and use the repeat pipettor to add 100 µL reconstituted analyte-specific Probe Reagent to each tube. All reaction mixtures should be yellow in color.
   b. Cover the tubes with the sealing card(s) and frame. Place the rack in the sample block and lock the knobs over the frame.
   c. Press the ▶ key to continue. The SB100 instrument will:
      i. Vortex the rack for 10 seconds.
      ii. Incubate the rack at 62°C for 20 minutes.
   d. When the incubation is over, the message on the screen indicates that the rack should be removed to cool.
      i. Remove the rack from the sample block and incubate at room temperature for 5 minutes.
      ii. Press the ▶ key to start the timer.

      Note: *The rack must be removed and the ▶ key pressed within 5 minutes of completion of the Probe incubation. Otherwise a Suspend Time error will occur. The error message indicates how much time has elapsed since it has been in error mode. Either continue or abort the protocol.*
   e. After 5 minutes the SB100 instrument will beep. The message on the screen indicates that Selection Reagent will be added next.
3. Selection
   a. Remove the sealing card(s) and using the repeat pipettor, add 250 µL Selection Reagent to each tube. All reaction mixtures should be pink in color.
   b. Cover the tubes with the sealing card(s), attach the frame, load the rack in the sample block, and lock the knobs over the frame.
   c. Press the ▶ key to continue. The SB100 instrument will:
      i. Vortex the rack for 10 seconds.
      ii. Incubate the rack at 62°C for 10 minutes.
      iii. Cool the rack to 23°C.
   d. When the Selection incubation is complete, the message on the screen indicates that the rack should be removed.
   e. Remove the rack and remove the frame, but leave the sealing card(s) on the tubes. The message on the screen indicates that the Post-Amp protocol is complete.
   f. Press any key to return to the **Main Menu**.
   g. Turn off the post-amp SB100 instrument(s) if there are no further tests to be run.
   h. Proceed with the steps under *Detection*.

J. Detection

For use of the LEADER HC+ Luminometer, refer to the *LEADER HC+ Luminometer Operator's Manual*. For use of the PROGENSA PCA3 Assay Software, refer to the *PROGENSA PCA3 Assay Software System Administrator's Manual* and *Operator's Manual*.

1. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the reactions.
2. Prepare the LEADER HC+ Luminometer by placing one empty TTU in cassette position number 1 and perform the WASH protocol once.
3. Load the TTUs into the luminometer using the diagram in the luminometer as a guide. If testing both analytes (back-to-back run), load all PCA3 TTUs first, immediately followed by all PSA TTUs.
4. Log on to the computer. Click **NEW RUN** and select the appropriate assay protocol and concentrations. Click **NEXT** to begin the run.

   Note: *The run must be completed within 2 hours of the end of the 62°C Selection incubation.*
5. Prepare Deactivation Fluid by mixing equal volumes of 5.25% (0.7 M) sodium hypochlorite solution and Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. This Deactivation Fluid is stable for 4 weeks at room temperature.
6. When the run is finished, the assay software will generate two run reports, a Raw Run Report and a Ratio Report, if the runs are back-to-back (see *Quality Control Procedures* and *Interpretation of Results*).
7. When the run is finished, remove the used TTUs from the luminometer and place the TTUs into the container of Deactivation Fluid. Allow the TTUs to sit in the container for at least 15 minutes before disposal. Proper handling and disposal methods should be established by the laboratory director.

Procedural Notes

A. Specimen Preparation

1. If specimens contain suspended precipitates, heat at 37°C for up to 5 minutes followed by gentle inversion. In the event that the precipitate does not go back into solution, ensure that the precipitate does not prevent delivery of specimen.
2. Frozen specimens must be thawed at room temperature (15°C to 30°C, may use a water bath) with occasional inversion during the thaw to prevent formation of an insoluble plug. Mix the vials by gentle inversion once the ice inside the vial has thawed enough to become loose and can move freely. Continue warming until the specimen is completely thawed and again mix the vials by gentle inversion.
   a. If a plug forms and specimens will be pipetted with the TECAN Freedom EVO 100/4 instrument, refreeze the specimen, repeat the thawing instructions and ensure that no plug forms. If unable to eliminate the plug, specimen must be hand-pipetted.
   b. If a plug forms and specimens will be hand-pipetted with a micropipettor, no further actions are necessary but ensure the plug does not prevent delivery of specimen.

B. Control, Calibrator, and Specimen Pipetting

1. The volume of calibrator, control, or specimen added to the TTU should be 400 µL. Visual inspection of the volume pipetted into the TTU is recommended to ensure proper volume transfer. Proper volume is needed to provide accurate results.
2. Ensure the pipette tip is seated correctly on the pipettor and check that the volume setting is correct. It is recommended to visually check the volume setting at the end of each TTU (every 10 tubes). Release the pipette plunger slowly at a steady rate when drawing the sample, to avoid generation of foam and bubbles.

C. Reagents

1. Probe Reconstitution Solution may precipitate during storage. Warm the solution at 62°C ± 1°C for 1 to 2 minutes. After this heat step, the Probe Reconstitution Solution may be used even if residual precipitate remains. After resuspension, mix the vial by gentle inversion.
2. When pipetting reagents other than Enzyme Reagent, aim slightly to the side of the bottom of the reaction tube (where the bottom curves up to meet the sides). When pipetting Enzyme Reagent, aim directly for the center of the reaction tube. Visually confirm that reagents are being dispensed correctly (no excessive amount of reagent on the sides of the tubes and proper color change).

D. Temperature

1. The target capture, amplification, hybridization, and selection steps are temperature dependent.
2. Room temperature is defined as 15°C to 30°C.

E. Time

The target capture, amplification, hybridization, and selection reactions are all time dependent. Adhere to specific times in the *DTS Systems Test Procedure*.

F. Decontamination

1. Surfaces and Pipettors

   Laboratory bench surfaces and pipettors must be decontaminated regularly with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the solution to contact surfaces for at least 1 minute and then follow with a water rinse. **Do not allow the sodium hypochlorite solution to dry.** Chlorine solutions may pit equipment and metal. Thoroughly rinse equipment with water to avoid pitting.

2. TCS Aspiration Manifold

   After each use:

   a. Move the dispense manifold out of the way.
   b. Place a new TTC into the TTC rack. Turn on the vacuum pump. Attach the aspiration manifold to the tips in the TTC. Aspirate all Wash Solution remaining in the priming trough of the Wash Solution dispense station.
   c. Pour at least 100 mL of 0.5% to 0.7% (0.07 M to 0.1 M), or if preferred 2.5% to 3.5% (0.35 M to 0.5 M), sodium hypochlorite solution into the priming trough. Aspirate all of the solution through the aspiration manifold.

d. Pour at least 100 mL of deionized water into the priming trough. Aspirate all of the water through the aspiration manifold.
e. Eject the tips into their original TTC.
f. Leave the vacuum pump on until the manifold tubing is dry to prevent back flow (about 3 minutes).
g. Decontaminate the aspiration manifold surfaces as described in *TCS Unit*.

3. TCS Waste Container

   Clean the waste bottle at least once a week or when the waste bottle is 25% full, whichever comes first.

   a. Turn off the vacuum pump and allow the vacuum pressure to equalize.
   b. Release the quick disconnect fittings between the waste bottle and overflow bottle, and the waste bottle and aspiration manifold.
   c. Remove the waste bottle from the vacuum trap enclosure.
   d. Remove the cap and carefully add 400 mL of 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution to the bottle (or 1 L if using a 10 L waste bottle).

      Note: *This may be done in a fume hood to avoid the release of fumes into the laboratory.*

   e. Cap the waste bottle and gently swirl the contents until fully mixed.
   f. Let the waste bottle sit for 15 minutes and then dispose of the contents (waste).
   g. Rinse the waste bottle with water to remove any remaining waste.
   h. Cap the empty waste bottle and place it in the vacuum trap enclosure. Attach the quick disconnect fitting to the TCS unit. Carefully discard both gloves.

4. TCS Unit

   Wipe the surfaces of the TCS unit, aspiration manifold, and surface of the Wash Buffer ejector tips with paper towels moistened with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Follow with a water rinse and then dry the surfaces completely with paper towels.

5. SB100 Instruments

   Do not spray any fluid directly on the instrument. There is wiring under the deck that will be damaged if sodium hypochlorite solution drips onto it. Do not pour or squirt sodium hypochlorite solution or water directly onto the SB100 instrument.

   Wipe the surfaces of the instrument (or frame) with paper towels moistened with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the solution to contact surfaces for at least 1 minute. After a minute, thoroughly wipe the surfaces with water-moistened paper towels to avoid pitting. Dry the surface completely with a paper towel.

6. Racks

   Submerge the racks in 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution, ensuring that they are covered by the solution. Keep the racks submerged for 10 minutes. Longer exposure will damage the racks. Rinse the racks thoroughly with water and then dry completely with paper towels.

G. Assay Contamination

1. The introduction of contaminating materials may occur if sufficient care is not taken during the assay procedure.
2. TTUs must be decontaminated in Deactivation Fluid as described in the *DTS Systems Test Procedure*. Do not reuse the TTUs.
3. Perform regular decontamination of equipment and work surfaces as described in *Decontamination*.
4. As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. It is recommended that operators use powderless gloves.

Quality Control Procedures

A. Run Validity

1. Calibrators and controls must be run with all assays and on the same rack as test specimens. The following criteria must be met in order for a run to be considered valid:

   Average RLU of Calibrator 2 > RLU Cutoff

   Where RLU Cutoff = Average RLU of Calibrator 1

   + 1.645 standard deviations of Calibrator 1 RLU replicates

   + 1.645 standard deviations of Calibrator 2 RLU replicates.

   Average interpolated Calibrator 5 recovery = 100 ± 30%

   Average interpolated Control A recovery = 100 ± 60%

   Average interpolated Control B recovery = 100 ± 35%

2. The PROGENSA PCA3 Assay Software automatically evaluates the results against the above criteria and will report the Run Status as PASS if the validity criteria are met, and FAIL if the validity criteria are not met.
3. If the Run Status is FAIL, all test results in the same run are invalid for that analyte and must not be reported.
4. If a run is invalid, the run must be repeated for that analyte (see *Interpretation of Results*). If the run is valid for the other analyte, those results may be used in data analysis with the repeat, valid run of the first analyte.

B. Specimen Validity

Within a valid run, individual specimen results may be deemed INVALID and will be indicated in the Raw Run Report (see *Interpretation of Results*). Although individual replicates for a specimen may be valid, a specimen will be invalidated if the interpolated copies/mL difference between the replicates exceeds 5.8-fold (the second replicate is 5.8 times more than the first replicate or the second replicate is 5.8 times less than the first replicate or coefficient of variation of the two replicates is more than 100%). Testing of the specimen for that analyte must be repeated.

C. External Controls

Each laboratory under their normal operating conditions should establish their own external controls to monitor test system components, environment, and operator performance. Previously tested patient specimens may be used, provided the laboratory determines the acceptable performance level for the patient specimens. The laboratory will establish the frequency of testing controls that detect immediate errors and monitor test performance over time and maintain records according to standard laboratory quality control practices (2, 3, 8).

Interpretation of Results

A. Types of Reports

1. Raw Run Report

   The Raw Run Report provides information on run validity (PASS or FAIL; see *Quality Control Procedures*) and on the individual reaction tubes tested with the PROGENSA PCA3 Assay. If a run is invalid (FAIL), all tubes in that run will be labeled invalid. However, individual tubes may be deemed invalid within a valid run (PASS). For back-to-back runs (i.e., both PCA3 and PSA analytes are tested in the same assay run), one analyte run may be invalid while the other analyte run is valid.

   The Exceptions Summary is found at the end of the Raw Run Report. For back-to-back runs where both analyte runs are valid, specimens listed in the Exceptions Summary may require retesting of one analyte. Although a PCA3 Score result may be listed in the Exceptions Summary, this result is not considered reportable until manual matching has been performed and the result is listed in a Ratio Report. If only one analyte was tested or if one analyte run is invalid, all specimens tested will be listed in the Exceptions Summary.

2. Ratio Report

   The assay software automatically generates a Ratio Report for a back-to-back run where both analyte runs are valid. The software calculates and lists the PCA3 Score of specimens in the Ratio Report. Specimens listed in the Ratio Report either require no further testing or both analytes must be retested. Specimens not listed in the Ratio Report will be found in the Exceptions Summary section of the Raw Run Report.

   A Ratio Report can also be generated after manual matching (see *Manual Matching* for more information).

3. QC Report

   The QC Report lists assay run validity criteria, assigned and interpolated concentrations, and recoveries of calibrators and controls. The report also lists the parameters that define the four-parameter logistic dose response calibration curve (9). For more information, refer to the *PROGENSA PCA3 Assay Software Operator's Manual.*

B. Matching

1. Automatic Matching

   In back-to-back runs where both analyte runs are valid, the software automatically matches the individual PCA3 and PSA analyte results for specimens and determines the PCA3 Score (if calculable). The results are listed in the Ratio Report or Exceptions Summary of the Raw Run Report.

2. Manual Matching

   When PCA3 and PSA analytes are tested in different runs, the software cannot automatically determine the PCA3 Score. Manual matching of the analyte results is necessary to determine the PCA3 Score or PCA3 Score range (refer to the *PROGENSA PCA3 Assay Software Operator's Manual*). Manual matching may also be required for results that are listed in the Exceptions Summary of the Raw

Run Report. After manual matching, the PCA3 Score(s) for the matched specimen(s) will be listed in a new Ratio Report.

C. Interpreting Reports

1. PCA3 Score

Note: Only PCA3 Scores and PCA3 Score ranges listed in the Ratio Report are reportable. *Results that appear in the Exceptions Summary may require further action and are not reportable.*

The PCA3 Score is calculated as the ratio of PCA3 RNA copies to PSA RNA copies, multiplied by 1000. PCA3 Scores may only be calculated using results from valid runs and specimens. Invalid runs and invalid specimens must be retested for that analyte (see *Retesting* for more information).

If the reported PCA3 Score is below the cut-off of 25, the result should be interpreted as NEGATIVE. If the PCA3 Score is above or equal to the cut-off of 25, the result should be interpreted as POSITIVE. A NEGATIVE result is associated with decreased likelihood of a positive biopsy. Due to normal assay variability, specimens with PCA3 Scores near the cut-off of 25 (i.e., 18 to 31) could yield a different overall interpretation of POSITIVE or NEGATIVE upon repeat testing. PCA3 Scores in the range from 18 to 31 should, therefore, be interpreted with caution.

For specimens with PCA3 analyte levels outside the calibrator range and PSA analyte levels inside the calibrator range, the PCA3 Score may be reported as a range (>[(125,000/B)*1000] or <[(250/B)*1000] where B is the PSA analyte level).

For specimens with PSA analyte levels above the calibrator range and PCA3 analyte levels inside the calibrator range, the PCA3 Score may be reported as a range <[(A/3,000,000)*1000] where A is the PCA3 analyte level.

Specimens with PSA analyte levels below the calibrator range have insufficient RNA for accurate analysis and a new specimen must be collected.

If <[Calculated Score] is below the cut-off of 25, the result should be interpreted as NEGATIVE. If >[Calculated Score] is above the cut-off of 25, the result should be interpreted as POSITIVE. In some cases, it may not be possible to determine if a specimen is POSITIVE or NEGATIVE. For example, if the PCA3 Score obtained is "<100", an overall interpretation relative to the cut-off of 25 cannot be made. If a numerical value is required for interpretation relative to the cut-off of 25, specimen dilution and retesting may generate a PCA3 Score instead of a PCA3 Score range. If dilution and retesting still cannot provide a PCA3 Score which can be used for interpretation relative to the cut-off of 25, another specimen collection must be requested.

2. Interpreting Status and Analysis Codes

The Status column in both the Raw Run Report and Ratio Report lists information in "s:a" format. Run-specific status codes ("s") are listed before (to the left of) the colon and analyte-specific analysis codes ("a") are listed after (to the right of) the colon. Analyte-specific codes are listed in lowercase for PCA3 results and uppercase for PSA results. Each report contains descriptions of the status and analysis codes that appear in that report. For example, codes may indicate if a specimen or replicate result is valid or out-of-range. Refer to the *PROGENSA*

PCA3 Assay Software Operator's Manual for a full listing of status and analysis codes and more details.

If a PCA3 Score is reported in the Ratio Report and no status or analysis codes appear in the PCA3 or PSA Status columns, this indicates both analytes tested valid and "in range." The specimen result is reportable and no further actions are necessary.

If a status or analysis code appears in the Exceptions Summary or in the Ratio Report, retesting may be necessary (see *Interpreting the Results in the Exceptions Summary* and *Interpreting Results in the Ratio Report*). If analyte results come from separate runs and have an analysis code(s), find the combination for both analytes in Table 4 or Table 5 to determine if further action is necessary.

3. Interpreting the Results in the Exceptions Summary

   The Exceptions Summary may not list any exceptions. In these cases, no further actions are necessary.

   If the Exceptions Summary lists a specimen(s) for back-to-back runs where both analyte runs are valid, refer to Table 4 for instructions.

   For individual analyte runs, refer to *Interpreting Status and Analysis Codes*. In back-to-back runs where one analyte run is invalid, retest the invalid run (see *Retesting* for more information), and treat the results as though individual analyte runs had been performed. Manual matching will be required.

   A specimen may be labeled as invalid although the individual tubes (replicates) may be labeled as valid. It is the combined result of the replicates that determines specimen validity, and a large difference between replicates will invalidate a specimen (see *Quality Control Procedures* for more information).

Table 4: PROGENSA PCA3 Assay Exceptions Summary Conditions

PCA3 Result (Analysis Code*)	PSA Result (Analysis Code*)	Listed PCA3 Score	Further Testing?	Action/Comment
In range (no code)	Invalid** (A, B, E, H, or I)	--	Yes	Retest PSA (see *Retesting*) and manually match results.
Out-of-range low (g)	Invalid (A, B, E, H, or I)	--	Yes	Retest PSA (see *Retesting*) and manually match results.
Invalid (a, b, e, h, or i)	In range (no code)	--	Yes	Retest PCA3 (see *Retesting*) and manually match results.
In range (no code)	Out-of-range high (F)	<[Calculated PCA3 Score]***	Optional	1. Manually match to get <[Calculated PCA3 Score] OR 2. Dilute specimen in specimen diluent (see *Dilution of Out-of-Range High Specimens*), retest PSA, and manually match results if a PCA3 Score is required.
Out-of-range high (f)	In range (no code)	>[Calculated PCA3 Score]	Optional	1. Manually match to get >[Calculated PCA3 Score] OR 2. Dilute specimen in specimen diluent, retest PCA3, and manually match results if a PCA3 Score is required.
Out-of-range low (g)	In range (no code)	<[Calculated PCA3 Score]	No	Manually match to get <[Calculated PCA3 Score].
Out-of-range low (g)	Out-of-range high (F)	<[Calculated PCA3 Score]	No	Manually match to get <[Calculated PCA3 Score].

*Refer to the *PROGENSA PCA3 Assay Software Operator's Manual* for a full listing of analysis codes.

**Applies only to invalid specimens within a valid run.

***For out-of-range values, the Calculated PCA3 Score is computed using the copy level for the nearest positive calibrator.

4. Interpreting Results in the Ratio Report

   If a specimen is listed in the Ratio Report with a PCA3 Score, the result is a reportable PCA3 Score and no further actions are necessary. If no PCA3 Score is listed, expressed as "--" in the PCA3 Score column, refer to Table 5 for instructions.

Table 5: PROGENSA PCA3 Assay Ratio Report Conditions

PCA3 Result (Analysis Code*)	PSA Result (Analysis Code*)	Listed PCA3 Score	Further Testing?	Action/Comment
In range (no code)	In range (no code)	PCA3 Score	No	No further actions; result is reportable.
Invalid** (a, b, e, h, or i)	Invalid (A, B, E, H, or I)	--	Yes	Retest both analytes (see *Retesting*).
Invalid (a, b, e, h, or i)	Out-of-range high (F)	--	Yes	Dilute specimen in specimen diluent (see *Dilution of Out-of-Range High Specimens*), retest both analytes.
Out-of-range high (f)	Invalid (A, B, E, H, or I)	--	Yes	Dilute specimen in specimen diluent, retest both analytes.
Out-of-range high (f)	Out-of-range high (F)	--	Yes	Dilute specimen in specimen diluent, retest both analytes.
Invalid (a, b, e, h, or i)	Out-of-range low (G)	--	No	Sample has insufficient RNA for accurate analysis. A new specimen must be collected from the patient.
In range (no code)	Out-of-range low (G)	--	No	Sample has insufficient RNA for accurate analysis. A new specimen must be collected from the patient.
Out-of-range high (f)	Out-of-range low (G)	--	No	Sample has insufficient RNA for accurate analysis. A new specimen must be collected from the patient.
Out-of-range low (g)	Out-of-range low (G)	--	No	Sample has insufficient RNA for accurate analysis. A new specimen must be collected from the patient.

*Refer to the *PROGENSA PCA3 Assay Software Operator's Manual* for a full listing of analysis codes.

**Applies only to invalid specimens within a valid run. If specimens were invalid because the run was invalid, results will be listed in the Exceptions Summary (see *Interpreting the Results in the Exceptions Summary* for more information).

D. Retesting

1. Guidelines for Retesting

a. Although it is not imperative that both analytes be tested in the same run, **both analyte results must come from the same sample vial for a reportable PCA3 Score**. In case the analytes are not run in the same run but are tested in different runs, the software cannot automatically determine the PCA3 Score. Manual matching of the analyte results is necessary to determine the PCA3 Score or PCA3 Score range (refer to the PROGENSA PCA3 Assay Software Operator's Manual)

b. All invalid runs must be repeated and all invalid specimens from valid runs must be retested.

c. Retest the specimen(s) using a new set of calibrators and controls.

d. Proper storage of the leftover specimen prior to retesting is essential (see *Specimen Collection, Transport, and Storage* for more information).

e. A manual match of PCA3 and PSA analytes may be necessary to determine the PCA3 Score (see *Manual Matching* for more information).

2. Dilution of Out-of-Range High Specimens
   a. If a specimen concentration extrapolates above Calibrator 5 within a valid run, the result is "out-of-range high" and the result will be labeled with an "f" or "F" analysis code in the run report(s). The concentration will be expressed as >[Calibrator 5 concentration].
   b. Invert the processed urine specimen to mix it prior to dilution of the specimen. A recommended, but not required, dilution is 1:10 using the PROGENSA PCA3 Specimen Diluent Kit. In an appropriate vial, add 1800 µL specimen diluent and 200 µL specimen; cap tube and invert five times to mix completely. The dilution factor will be "10" in the run worklist. If both analytes are to be retested, double the volumes (use 3600 µL specimen diluent and 400 µL specimen). Test the diluted specimen with the assay.
   c. If, upon retesting, the specimen result is again out-of-range high, further dilution until the specimen result interpolates within range of the calibrators is required. Further dilution of the initial 1:10 dilution is permissible, provided the initial 1:10 dilution was stored properly (see *Specimen Collection, Transport, and Storage* for more information).

Performance Characteristics

Clinical Performance

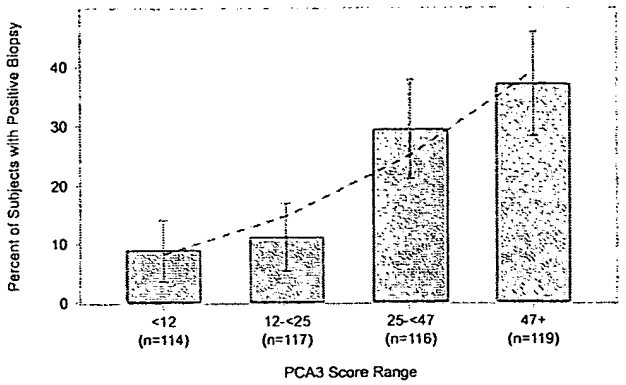
Four hundred ninety five (495) eligible male subjects were enrolled from a total of 14 clinical sites, including academic institutions, community-based urology clinics, and group health organizations. Men who had at least one previous negative prostatic biopsy, who had never had a positive prostatic biopsy, and who had been recommended for a repeat biopsy by their urologists were eligible for study participation. A questionnaire was used to collect information regarding prostate cancer risk factors from men recommended for a repeat biopsy by their clinician (i.e., the enrolled population) and from men not recommended for a repeat biopsy (i.e., the non-enrolled population). Age, prostate volume, and most recent free PSA test result were not significantly different between enrolled and non-enrolled populations. Serum PSA test results and the time since the most recent negative biopsy were significantly different ($P<.0001$) between enrolled and non-enrolled populations, where the non-enrolled men (men not recommended for repeat biopsy by their clinician) had 2.2 ng/mL lower mean serum PSA test results and approximately 60% shorter time since their most recent previous negative biopsy. Clinical study analysis included men who had been recommended for a repeat biopsy by their urologists.

Blood, urine, and prostatic biopsy specimens were collected from each subject enrolled in the study. The blood specimen was tested with a total serum PSA test at the collection site's associated testing facility. The total serum PSA test used varied by collection site. The urine specimen was collected following a digital rectal exam (DRE) and was a first-catch urine specimen. The urine specimen was processed at the collection site by aliquotting into PROGENSA PCA3 Urine Specimen Transport Tubes and shipped to a testing site for PROGENSA PCA3 Assay testing. The prostatic biopsy was performed per the collection site's standard procedure. The biopsy specimens were evaluated by the collection site's associated pathology facility(ies).

For the 495 eligible subjects, the median age was 67.0 years; ages ranged from 44 years to 92 years. Race was reported as White for 433 subjects (87.5%), Black or African American for 45 subjects (9.1%), Asian for 11 subjects (2.2%), American Indian/Alaska Native for 2 subjects (0.4%), and unknown for 5 subjects (1.0%); one subject reported both White and American Indian/Alaska Native. Four hundred and eighty (480) of the eligible subjects provided a urine sample for PROGENSA PCA3 Assay testing (3.0% (15/495) of subjects did not provide a urine sample); 1.3% (6/480) of sample results were excluded because of sample qualification failure (insufficient RNA for accurate analysis), leaving 474 subjects with a valid and reportable PCA3 Score.

Four hundred and sixty-six (466) subjects with valid and reportable PCA3 Scores and disease status (determined by biopsy result), and who were 50 years of age or older were included in the analyses. Prevalence of positive biopsy was 21.9% (102/466). For the subjects with a study total serum PSA test result (n=464), the median total serum PSA test result was 5.80 ng/mL (results ranged from 0.3 ng/mL to 49.2 ng/mL). Prostatic biopsies consisted of 6 to 24 cores with 93% of subjects having 12 to 21 cores taken.

Figure 3 shows the percentage of subjects with positive prostatic biopsy results by PCA3 Score interval (with 95% confidence limits).



Note. Dashed line represents the predicted probability of positive biopsy from a logistic regression model. Ranges represent quartiles of the PCA3 Score distribution.

Figure 3. Positive Biopsy Results by PCA3 Score with 95% Confidence Limits

Table 6 shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome at a PCA3 Score cut-off value of 25.

Table 6: Performance Characteristics of the PROGENSA PCA3 Assay

	Biopsy Result		Total	Performance Characteristic	Estimate	95% CI
	Biopsy Positive	Biopsy Negative				
PCA3 Score ≥25	79	156	235	**Sensitivity %**	77.5 (79/102)	68.4-84.5
PCA3 Score <25	23	208	231	**Specificity %**	57.1 (208/364)	52.0-62.1
Total	102	364	466	**PPV %**	33.6 (79/235)	30.0-37.2
				NPV %	90.0 (208/231)	86.5-93.1
				PLR	1.81	1.53-2.11
Positive Biopsy Prevalence %	21.9 (102/466)			**NLR**	0.40	0.26-0.56
				Odds Ratio	4.58	2.75-7.62

CI = confidence interval, PPV = positive predictive value, NPV = negative predictive value, PLR = positive likelihood ratio, NLR = negative likelihood ratio.

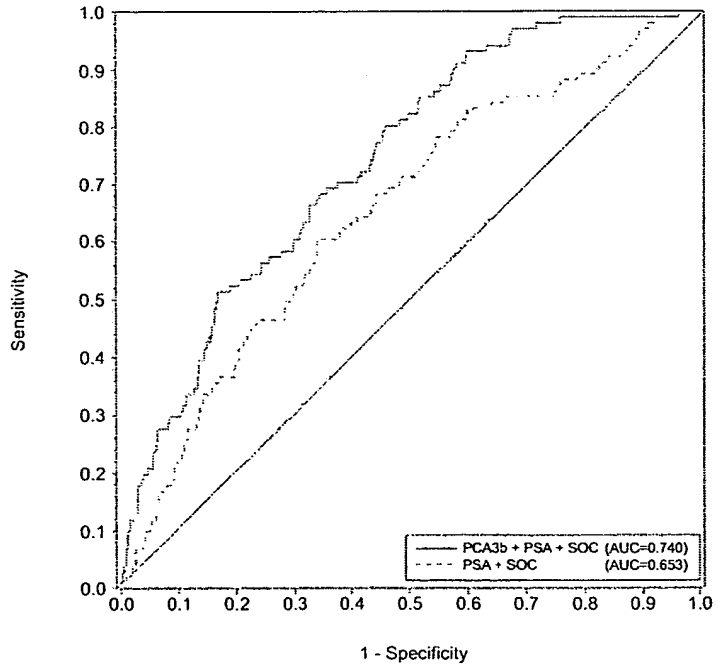
Table 7 shows the area under the curve (AUC) of the receiver operating characteristic (ROC) curves for the PROGENSA PCA3 Assay. The ROC AUC for PCA3 Score was 0.707 (95% CI: 0.649–0.746). The ROC AUC for total serum PSA test combined with standard of care covariates (including age, DRE result, family history of prostate cancer, race, and number of previous negative biopsy procedures; multivariable logistic regression model "PSA + SOC") was 0.653 (95% CI: 0.593–0.713). When PCA3 Score of 25 as a binary decision point was added to PSA + SOC, the ROC AUC was 0.740 (95%CI: 0.689–0.791); an increase of 0.087 (95% CI: 0.037–0.137).

Table 7: Receiver Operating Characteristics of the PROGENSA PCA3 Assay, Total Serum PSA Test, and Standard of Care Covariates

Model	ROC AUC (95% CI)	ROC AUC Comparison	ROC AUC Difference (95% CI)[1]
PCA3c	0.707 (0.649 - 0.764)	N/A	N/A
PSA + SOC	0.653 (0.593 - 0.713)	N/A	N/A
PCA3b + PSA + SOC	0.740 (0.689 - 0.791)	(PCA3b + PSA + SOC) - (PSA + SOC)	0.087 (0.037 - 0.137)
PCA3c + PSA + SOC	0.733 (0.679 - 0.786)	(PCA3c + PSA + SOC) - (PSA + SOC)	0.080 (0.033 - 0.126)
PCA3c + PSA	0.710 (0.653 - 0.766)	(PCA3c + PSA) - PSA	0.105 (0.042 - 0.168)

PCA3c = PCA3 Score (continuous), PCA3b = PCA3 Score using a binary test with cut-off of 25, PSA = total serum PSA test, SOC = standard of care covariates, AUC = Area Under the ROC curve, N/A = not applicable,CI = confidence interval.
[1]AUC for the PROGENSA PCA3 Assay minus the AUC for the comparator.

Figure 4 shows the ROC curves for total serum PSA test results and standard of care covariates with and without PROGENSA PCA3 Assay results using a PCA3 Score of 25 as a cut-off.



PCA3b = PCA3 Score using a binary decision point of 25, PSA = total serum PSA test, SOC = standard of care covariates.

Figure 4. ROC Curves for Total Serum PSA Test Results and Standard of Care Covariates, with and without PCA3 Score with Cut-off of 25

Table 8 shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome at selected cut-offs.

Table 8: Performance Characteristics of the PROGENSA PCA3 Assay at Selected Cut-offs

PCA3 Score Cut-off	n	TP	FP	TN	FN	Se % (95% CI)	Sp % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	PLR (95% CI)	NLR (95% CI)	OR (95% CI)
5	466	100	343	21	2	98.0 (93.1-99.5)	5.8 (3.8-8.7)	22.6 (21.7-23.2)	91.3 (74.3-99.1)	1.04 (0.99-1.08)	0.34 (0.03-1.23)	3.06 (0.71-13.27)
10	466	94	279	85	8	92.2 (85.3-96.0)	23.4 (19.3-28.0)	25.2 (23.6-26.7)	91.4 (84.8-95.9)	1.20 (1.10-1.30)	0.34 (0.15-0.64)	3.58 (1.67-7.66)
15	466	87	233	131	15	85.3 (77.1-90.9)	36.0 (31.2-41.0)	27.2 (24.9-29.4)	89.7 (84.7-93.7)	1.33 (1.18-1.49)	0.41 (0.24-0.64)	3.26 (1.81-5.87)
20	466	80	188	176	22	78.4 (69.5-85.3)	48.4 (43.3-53.5)	29.9 (26.8-32.8)	88.9 (84.8-92.4)	1.52 (1.31-1.75)	0.45 (0.29-0.64)	3.40 (2.04-5.70)
25	**466**	**79**	**156**	**208**	**23**	**77.5 (68.4-84.5)**	**57.1 (52.0-62.1)**	**33.6 (30.0-37.2)**	**90.0 (86.5-93.1)**	**1.81 (1.53-2.11)**	**0.40 (0.26-0.56)**	**4.58 (2.75-7.62)**
35	466	64	112	252	38	62.7 (53.1-71.5)	69.2 (64.3-73.8)	36.4 (31.3-41.4)	86.9 (83.9-89.8)	2.04 (1.63-2.52)	0.54 (0.41-0.69)	3.79 (2.40-6.00)
45	466	46	79	285	56	45.1 (35.8-54.8)	78.3 (73.8-82.2)	36.8 (30.1-43.6)	83.6 (81.1-86.2)	2.08 (1.54-2.76)	0.70 (0.57-0.83)	2.96 (1.87-4.71)
55	466	38	63	301	64	37.3 (28.5-46.9)	82.7 (78.5-86.2)	37.6 (29.8-45.7)	82.5 (80.3-84.8)	2.15 (1.52-3.00)	0.76 (0.64-0.88)	2.84 (1.75-4.61)
65	466	36	39	325	66	35.3 (26.7-44.9)	89.3 (85.7-92.1)	48.0 (38.1-57.9)	83.1 (81.2-85.3)	3.29 (2.20-4.91)	0.73 (0.61-0.83)	4.55 (2.69-7.68)
75	466	34	34	330	68	33.3 (24.9-42.9)	90.7 (87.2-93.2)	50.0 (39.4-60.5)	82.9 (81.0-85.1)	3.57 (2.32-5.47)	0.74 (0.63-0.84)	4.85 (2.82-8.35)
100	466	24	22	342	78	23.5 (16.4-32.6)	94.0 (91.0-96.0)	52.2 (38.7-65.4)	81.4 (79.9-83.3)	3.89 (2.25-6.75)	0.81 (0.72-0.90)	4.78 (2.55-8.97)

TP = true positive, FP = false positive, TN = true negative, FN = false negative, CI = confidence interval, Se = sensitivity, Sp = specificity, PPV (NPV) = positive (negative) predictive value, PLR (NLR) = positive (negative) likelihood ratio, OR = odds ratio. For calculations in this table, PCA3 Score values greater than or equal to the cut-off are considered positive and PCA3 Score values less than the cut-off are considered negative.

Table 9 shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome for subgroups of the study population. Table 10 shows PROGENSA PCA3 Assay performance results in the subgroup of men with ASAP on their most recent negative biopsy. The clinical study was not designed to evaluate subgroups, so the results for individual subgroups may not be conclusive. However, PROGENSA PCA3 Assay performance in men with prior ASAP indicated that the PROGENSA PCA3 Assay is not informative of biopsy outcome in this subgroup (Table 10).

Table 9: Performance Characteristics of the PROGENSA PCA3 Assay by Subgroups

Subgroup	n	TP	FP	TN	FN	Se % (95% CI)[1]	Sp % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	PLR (95% CI)	NLR (95% CI)	OR (95% CI)
Age (years)												
50-59	96	10	19	59	8	55.6 (33.7-75.4)	75.6 (65.1-83.8)	34.5 (20.8-48.0)	88.1 (82.2-93.5)	2.28 (1.14-4.00)	0.59 (0.30-0.94)	3.88 (1.34-11.25)
60-69	193	24	62	94	13	64.9 (48.8-78.2)	60.3 (52.4-67.6)	27.9 (21.4-34.2)	87.9 (82.7-92.5)	1.63 (1.15-2.19)	0.58 (0.34-0.88)	2.80 (1.33-5.91)
70+	177	45	75	55	2	95.7 (85.8-98.8)	42.3 (34.2-50.9)	37.5 (33.6-41.6)	96.5 (89.5-99.5)	1.66 (1.40-1.97)	0.10 (0.02-0.33)	16.50 (3.84-70.94)
Prior Negative Biopsy Result												
HGPIN (not ASAP)	101	21	42	35	3	87.5 (69.0-95.7)	45.5 (34.8-56.5)	33.3 (27.1-39.5)	92.1 (81.7-98.2)	1.60 (1.20-2.09)	0.28 (0.06-0.72)	5.83 (1.61-21.20)
None/Other	316	48	90	163	15	76.2 (64.4-85.0)	64.4 (58.4-70.1)	34.8 (29.8-39.8)	91.6 (87.8-94.7)	2.14 (1.71-2.65)	0.37 (0.22-0.56)	5.80 (3.07-10.93)
Number of Previous Negative Biopsies												
1	316	56	101	138	21	72.7 (61.9-81.4)	57.7 (51.4-63.8)	35.7 (31.0-40.4)	86.8 (82.2-90.9)	1.72 (1.39-2.10)	0.47 (0.31-0.67)	3.64 (2.07-6.40)
2+	150	23	55	70	2	92.0 (75.0-97.8)	56.0 (47.2-64.4)	29.5 (24.3-34.6)	97.2 (91.9-99.6)	2.09 (1.61-2.65)	0.14 (0.02-0.44)	14.64 (3.31-64.78)
Timing of Previous Biopsy Relative to Study Enrollment												
<3 months[1]	13	2	6	2	3	40.0 (11.8-76.9)	25.0 (7.1-59.1)	25.0 (3.2-49.9)	40.0 (6.9-76.2)	0.53 (0.05-1.59)	2.40 (0.50-21.76)	0.22 (0.02-2.45)
3 months to <7 years	438	75	145	199	19	79.8 (70.6-86.7)	57.8 (52.6-63.0)	34.1 (30.4-37.8)	91.3 (87.7-94.3)	1.89 (1.60-2.22)	0.35 (0.22-0.51)	5.42 (3.14-9.36)
7+ years	15	2	5	7	1	66.7 (20.8-93.9)	58.3 (32.0-80.7)	28.6 (6.5-54.8)	87.5 (66.6-99.5)	1.60 (0.28-4.85)	0.57 (0.02-2.01)	2.80 (0.20-40.06)
Race												
Black	39	6	16	16	1	85.7 (48.7-97.4)	50.0 (33.6-66.4)	27.3 (14.3-37.9)	94.1 (79.4-99.8)	1.71 (0.76-2.79)	0.29 (0.01-1.19)	6.00 (0.65-55.66)
Non-Black	427	73	140	192	22	76.8 (67.4-84.2)	57.8 (52.5-63.0)	34.3 (30.5-38.1)	89.7 (86.0-92.9)	1.82 (1.53-2.15)	0.40 (0.27-0.57)	4.55 (2.69-7.69)
Serum PSA (ng/mL) and Digital Rectal Exam												
PSA <4 and DRE Norm	81	13	37	28	3	81.3 (57.0-93.4)	43.1 (31.8-55.2)	26.0 (18.8-32.3)	90.3 (78.5-97.6)	1.43 (0.94-1.94)	0.44 (0.10-1.11)	3.28 (0.85-12.62)
PSA ≥4 or DRE Abn	383	65	119	179	20	76.5 (66.4-84.2)	60.1 (54.4-65.5)	35.3 (31.1-39.6)	89.9 (86.1-93.3)	1.92 (1.58-2.30)	0.39 (0.25-0.56)	4.89 (2.81-8.49)
Serum PSA (ng/mL) and Number of Previous Negative Biopsies												
PSA >10 and 1 Bx	34	10	9	14	1	90.9 (62.3-98.4)	60.9 (40.8-77.8)	52.6 (38.4-68.4)	93.3 (73.4-99.8)	2.32 (1.31-4.53)	0.15 (0.01-0.76)	15.55 (1.69-143.16)
PSA ≤10 or 2+ Bx	430	68	147	193	22	75.6 (65.8-83.3)	56.8 (51.5-61.9)	31.6 (27.9-35.3)	89.8 (86.1-93.0)	1.75 (1.46-2.06)	0.43 (0.29-0.61)	4.06 (2.40-6.87)

TP =true positive, FP = false positive, TN = true negative, FN = false negative, CI = confidence interval, Se = sensitivity, Sp = specificity, PPV (NPV) = positive (negative) predictive value, PLR (NLR) = positive (negative) likelihood ratio, OR = odds ratio, Bx = biopsy. For calculations in this table, PCA3 Score values ≥25 are considered positive and values <25 are considered negative.

[1]In this subgroup, 84.6% (11/13) had ASAP on their most recent negative biopsy. In the clinical study, the PROGENSA PCA3 Assay was not predictive of repeat biopsy outcome in men with prior ASAP (Table 10).

Table 10: Performance Characteristics of the PROGENSA PCA3 Assay in Men with ASAP on Their Most Recent Negative Biopsy[1]

	Biopsy Result		Total	Performance Characteristic	Estimate	95% CI
	Biopsy Positive	Biopsy Negative				
PCA3 Score ≥25	10	24	34	**Sensitivity %**	66.7 (10/15)	41.7-84.8
PCA3 Score <25	5	10	15	**Specificity %**	29.4 (10/34)	16.8-46.2
Total	15	34	49	**PPV %**	29.4 (10/34)	19.1-38.2
				NPV %	66.7 (10/15)	44.7-87.0
				PLR	0.94	0.54-1.40
Positive Biopsy Prevalence %	30.6 (15/49)			**NLR**	1.13	0.34-2.80
				Odds Ratio	0.83	0.23-3.07

CI = confidence interval, PPV = positive predictive value, NPV = negative predictive value, PLR = positive likelihood ratio, NLR = negative likelihood ratio.

[1]The PROGENSA PCA3 Assay should not be used for men with atypical small acinar proliferation (ASAP) on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.

Precision: Reproducibility

PROGENSA PCA3 Assay reproducibility (5) was evaluated on DTS Systems at 3 external clinical testing sites using a 3-member reproducibility panel. Testing was performed using 3 reagent lots and 3 calibrator and control lots. Two operators at each of the 3 testing sites performed, over 15 days, 5 PROGENSA PCA3 Assay runs per each of the 3 reagent lots (1 lot per day). Each run contained 4 sets of the 3 reproducibility panel members. The total number of results for each panel member was 360.

Reproducibility panel members were created by spiking PCA3 and PSA *in vitro* transcripts into a urine matrix composed of negative (female) urine specimens and PROGENSA PCA3 Urine Transport Medium. The analyte concentrations and targeted PCA3 Scores for each panel member are shown in Table 11. Panel members 2 and 3 had RNA concentrations representative of the copy levels found in post-DRE urine specimens; Panel member 1 had RNA concentrations near the low end of the PCA3 and PSA dynamic ranges.

Table 11: Reproducibility Panel Composition

Panel Member	PCA3 RNA Concentration	PSA RNA Concentration	Targeted PCA3 Score
1	Low	Low	35
2	Mid	High	10
3	High	Mid	86

Table 12 summarizes the variability of the PROGENSA PCA3 Assay within runs, between runs, between sites/instruments, between operators, and between reagent lots for each panel member for PCA3 and PSA analyte copies/mL and for PCA3 Score.

Table 12: Reproducibility of the PROGENSA PCA3 Assay PCA3 Copies/mL, PSA Copies/mL, and PCA3 Score Results on DTS Systems by Panel Member

Parameter Panel Member	PCA3 Conc	PSA Conc	n[1]	Mean Value	Within Run		Between Run		Between Site		Between Operator		Between Lot		Total	
					SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
PCA3 copies/mL																
1	Low	Low	359	678	83	12.2	73	10.7	18	2.6	0	0.0	33	4.9	116	17.2
2	Mid	High	359	18,969	959	5.1	820	4.3	261	1.4	0	0.0	0	0.0	1,289	6.8
3	High	Mid	357	97,006	4,620	4.8	3,797	3.9	2886	3.0	2,246	2.3	3,237	3.3	7,721	8.0
PSA copies/mL																
1	Low	Low	359	16,747	2,713	16.2	1,621	9.7	0	0.0	568	3.4	318	1.9	3,226	19.3
2	Mid	High	359	1,638,117	127,184	7.8	117,100	7.1	45,603	2.8	0	0.0	68,132	4.2	191,337	11.7
3	High	Mid	357	994,851	65,724	6.6	69,177	7.0	33,705	3.4	0	0.0	26,336	2.6	104,569	10.5
PCA3 Score																
1	Low	Low	359	41	7.0	17.0	6.6	16.1	0.0	0.0	1.8	4.3	3.0	7.4	10.3	25.0
2	Mid	High	359	11	1.2	10.7	1.0	9.1	0.3	2.9	0.0	0.0	0.5	4.3	1.7	15.0
3	High	Mid	357	98	8.5	8.6	7.9	8.1	0.0	0.0	2.9	3.0	1.9	1.9	12.1	12.3

Conc = concentration.
[1]Five samples (1 sample of Panel Member 1, 1 sample of Panel Member 2, and 3 samples of Panel Member 3) had invalid or out-of-range PCA3 and/or PSA analyte results leading to invalid or non-evaluable PCA3 Scores and were not included in the analyses.

Precision: Repeatability

PROGENSA PCA3 Assay repeatability (5) was evaluated at Gen-Probe Incorporated using a 4-member repeatability panel. Three panel members (1 to 3) comprised PCA3 and PSA *in vitro* transcripts in processed female urine, similar to the reproducibility panels (see above). The fourth panel member comprised PCA3 and PSA *in vitro* transcripts in processed female urine diluted in specimen diluent.

Testing was performed using 1 reagent lot and 1 calibrators and controls lot. One operator performed 20 PROGENSA PCA3 Assay runs on DTS Systems; each run contained 4 sets of the 4 repeatability panel members. Table 13 summarizes the variability of the PROGENSA PCA3 Assay within runs, between runs and between days for each panel member for PCA3 and PSA analyte copies/mL and for PCA3 Score.

Table 13: PROGENSA PCA3 Assay Repeatability

Parameter Panel Member	PCA3 Conc	PSA Conc	n[1]	Mean	Within Run		Between Run		Between Day		Total	
					SD	CV%	SD	CV%	SD	CV%	SD	CV%
PCA3 copies/mL												
1	Low	Low	80	661	85	12.9	54	8.1	67	10.1	121	18.3
2	Mid	High	80	18,626	1,033	5.5	752	4.0	156	0.8	1,287	6.9
3	High	Mid	80	99,846	3,820	3.8	1,111	1.1	3,260	3.3	5,143	5.2
4	Mid/Diln	Mid/Diln	80	24,482	1,169	4.8	1,047	4.3	0	0	1,569	6.4
PSA copies/mL												
1	Low	Low	80	18,298	2,862	15.6	837	4.6	275	1.5	2,995	16.4
2	Mid	High	77	2,017,466	190,359	9.4	27,935	1.4	0	0	192,398	9.5
3	High	Mid	80	1,247,896	228,984	18.3	0	0	44,626	3.6	233,292	18.7
4	Mid/Diln	Mid/Diln	80	603,427	108,192	17.9	32,253	5.3	0	0	112,897	18.7
PCA3 Score												
1	Low	Low	80	36	6.9	19.0	2.8	7.7	0.9	2.3	7.5	20.7
2	Mid	High	77	9	1.1	12.0	0.6	6.2	0.1	0.6	1.2	13.6
3	High	Mid	80	81	11.1	13.6	0	0	2.7	3.3	11.4	14.0
4	Mid/Diln	Mid/Diln	80	41	6.0	14.6	3.8	9.3	0	0	7.1	17.3

[1]Three samples of Panel Member 2 had out-of-range PSA analyte results leading to non-evaluable PCA3 Scores and were not included in the analyses.

Analytical Specificity

A. Unspliced Transcript

PROGENSA PCA3 Assay was designed to detect only the prostate cancer-specific exon 3-exon 4 spliced PCA3 RNA (14). The assay did not detect 1.25 x 10^6 copies/mL of unspliced PCA3 RNA in processed female urine significantly above background.

B. Interfering Substances

The substances listed in Table 14 were added to aliquots of pooled clinical specimens. The specimens were tested with PROGENSA PCA3 Assay according to CLSI EP7-A2 (2005) (7). At the concentrations listed, no assay interference (no significant change in PCA3 Score) was observed.

Table 14: Substances Tested for PROGENSA PCA3 Assay Interference

Medications and Supplements		Endogenous Substances	
Substance	**Test Concentration**	**Substance**	**Test Concentration**
Acetaminophen	1.324 mmol/L	Albumin	60 g/L
Acetylsalicylic acid	3.62 mmol/L	Bilirubin (unconjugated)	0.342 mmol/L
Alfuzosin	30 mg/L	Calcium	5 mmol/L
Allopurinol	0.294 mmol/L	Cholesterol	13 mmol/L
Amlodipine	0.245 µmol/L	Glucose	55 mmol/L
Atenolol	37.6 µmol/L	Hemoglobin	2 g/L
Atorvastatin	25 mg/L	Immunoglobulin G	32 mg/L
Ciprofloxacin	30.2 µmol/L	Triglycerides	37 mmol/L
Diphenhydramine	19.6 µmol/L	Uric acid	1.4 mmol/L
Doxazosin	1.33 µmol/L	Red blood cells	5.10×10^7 cells/L
Doxycycline	67.5 µmol/L	White blood cells	7.60×10^7 cells/L
Dutasteride	1.5 mg/L		
Esomeprazole	0.12 g/L	**Microorganisms**	
Finasteride	15 mg/L	**Organism**	**Test Concentration**
Fluoxetine	11.2 µmol/L	*Candida albicans*	5×10^6 CFU*/L
Flutamide	2.25 g/L	*Escherichia coli*	5×10^6 CFU/L
Furosemide	0.181 mmol/L	*Klebsiella pneumoniae*	5×10^6 CFU/L
Ibuprofen	2.425 mmol/L	*Proteus mirabilis*	5×10^6 CFU/L
Levofloxacin	48.6 µmol/L	*Pseudomonas aeruginosa*	5×10^6 CFU/L
Lisinopril	0.74 µmol/L	*Staphylococcus aureus*	5×10^6 CFU/L
Metformin	0.31 mmol/L		
Selenium	1 mg/L		
Saw palmetto	11.25 g/L		
Sildenafil	12.9 nmol/L		
Sulfasalazine	0.754 mmol/L		
Tamsulosin	1.2 µg/L		
Terazosin	7.8 µmol/L		

*CFU = colony-forming units.

Analytical Sensitivity

The limit of quantitation of PROGENSA PCA3 Assay was determined using an 8-member analytical sensitivity panel. The panel comprised 4 blank specimens (processed female urine that contains no detectable prostate-specific PCA3 or PSA RNA) and the blank specimens each spiked with PCA3 and PSA *in vitro* transcripts at Calibrator 2 concentrations. One operator performed 10 PROGENSA PCA3 Assay runs on DTS Systems; each run contained 2 sets of the 8 analytical sensitivity panel members. The limit of detection and limit of quantitation were calculated according to CLSI EP17-A (6). The limit of detection of the PCA3 analyte was 239 copies/mL (CV 31.2%), and for the PSA analyte it was 3,338 copies/mL (CV 24.2%). The limits of quantitation of both analytes were the same as the corresponding limits of detection. The lower limit of the dynamic range of the PROGENSA PCA3 Assay is defined by the lowest positive calibrator.

Linearity and Measuring Intervals

Linearity Studies using PCA3 and PSA *in vitro* Transcripts in Processed Female Urine

The linear range of the PROGENSA PCA3 Assay was determined using an 11-member linearity panel. A dilution series was prepared from PCA3 and PSA *in vitro* transcripts in processed female urine due to unavailability of very high concentration clinical material. Dilutions spanned beyond the assay range for each analyte.

One operator performed 4 PROGENSA PCA3 Assay runs on DTS Systems; each run contained 2 sets of the 11-member linearity panel. Results were analyzed using regression analysis according to CLSI EP6-A (4). Results of weighted linear regression analysis are presented in Figure 5 .

For PCA3 analyte, the PROGENSA PCA3 Assay demonstrated linearity from 135 to 200,032 copies/mL with deviation from linearity less than 9% in this interval; the dynamic range of the assay for PCA3 analyte is 250 to 125,000 copies/mL. For PSA analyte, linearity was demonstrated from 4,670 to 3,874,323 copies/mL with deviation from linearity less than 7%; the dynamic range is 7,500 to 3,000,000 copies/mL.

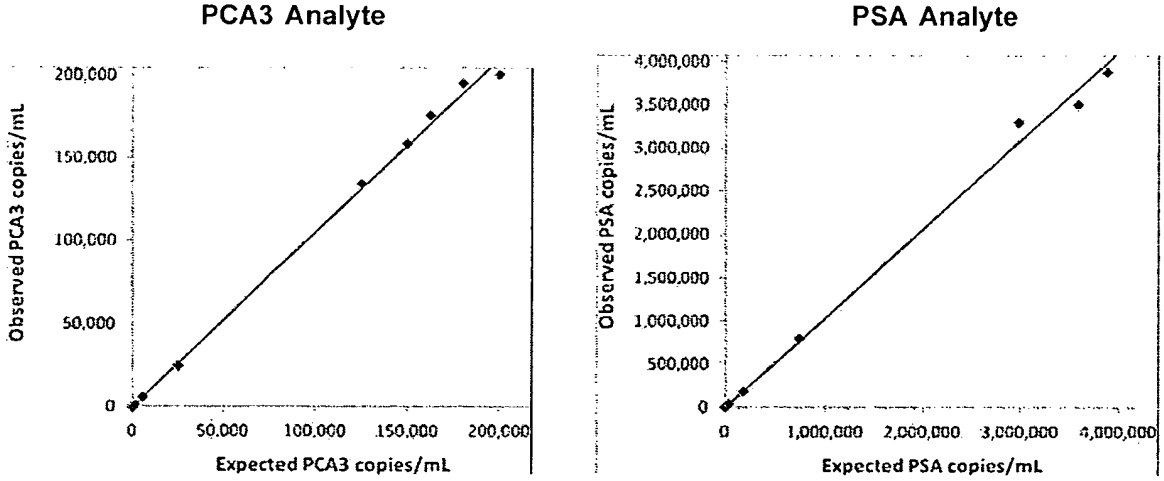


Figure 5. PROGENSA PCA3 Assay Linearity for PCA3 and PSA Analytes, Transcript Samples

Linearity Studies Using Clinical Specimens in Specimen Diluent or Processed Female Urine

Linearity was verified through 85% of the dynamic range in a dilution series of clinical materials. Two 10-member linearity panels, one each prepared in PCA3 Specimen Diluent or processed female urine independently for each analyte, were tested and analyzed as above. For PCA3 analyte, the PROGENSA PCA3 Assay demonstrated linearity from 130 to 104,564 copies/mL in processed female urine with deviation from linearity less than 6%. In PCA3 Specimen Diluent, the PCA3 analyte demonstrated linearity from 162 to 118,237 copies/mL with deviation less than 6% in this interval. For PSA analyte, linearity was demonstrated from 4,243 to 2,324,179 copies/mL with deviation from linearity less than 30%* in processed female urine. In PCA3 Specimen Diluent, the PSA analyte demonstrated linearity from 4,890 to 2,640,820 copies/mL in PCA3 Specimen Diluent with deviation less than 23%[1] in this interval. There was no significant diluent matrix effect. See Figure 6 (the solid line with triangles represents panel members diluted in processed

female urine, and the dashed line with circles represents panel members diluted in PCA3 Specimen Diluent).

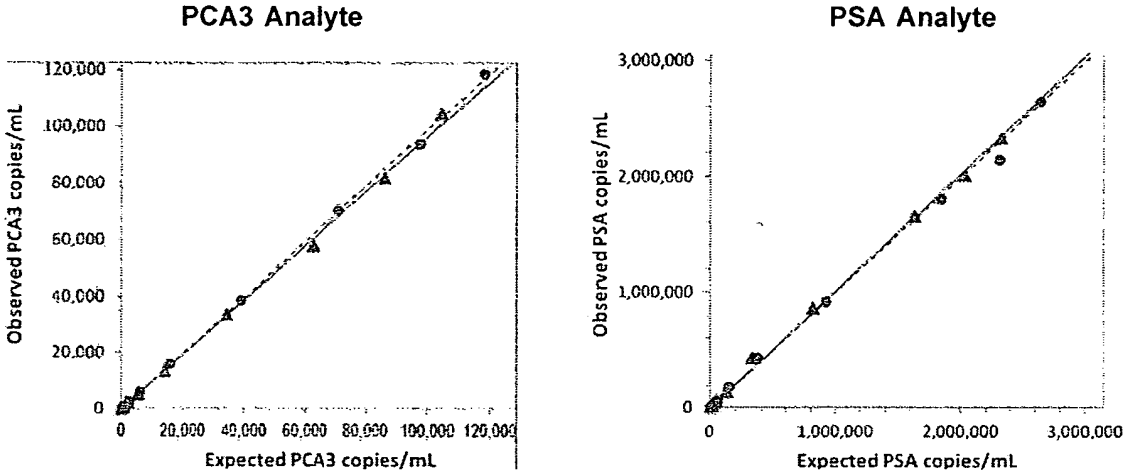


Figure 6. PROGENSA PCA3 Assay Linearity for PCA3 and PSA Analytes, Clinical Specimens

The measuring interval for PCA3 analyte is 250 to 125,000 copies/mL and the measuring interval for PSA analyte is 7,500 to 3,000,000 copies/mL. The interval of possible numerical values of the PCA3 Score is 0 to 16,667. In the clinical study, the range of the PCA3 Scores of 466 patients was 0 to 462.

Recovery

PROGENSA PCA3 Assay analyte quantitation was compared to an independent method (trueness could not be evaluated as no reference method yet exists). PCA3 and PSA *in vitro* transcripts were quantified by UV-vis spectrophotometry (assuming 1 optical density unit at 260nm is equal to 40 µg/mL RNA) at a much higher concentration than tested with PROGENSA PCA3 Assay. An 8-member test panel was prepared by dilution of the UV-quantified transcripts into processed female urine (10^7- to 10^{10}-fold). Two operators each performed 4 PROGENSA PCA3 Assay runs; each run contained 4 sets/replicates of the 8-member test panels. Percent recovery was calculated as the ratio of PROGENSA PCA3 Assay measured copies/mL to UV-determined copies/mL, multiplied by 100 (Table 15).

1 Although the deviation from linearity for the PSA analyte was within study acceptance criteria, the higher-than-expected deviation may have been caused by variation during linearity panel preparation.

Table 15: Copy Recovery of the PROGENSA PCA3 Assay

Analyte	Panel Member	n[1]	UV-Calculated Concentration, copies/mL	Measured Concentration, copies/mL	Recovery
PCA3	1	32	1,250	1,377	110%
	2	32	12,500	12,452	100%
	3	32	62,500	56,501	90%
	4	32	6,250	7,244	116%
	5	32	250	294	118%
	6	32	500	590	118%
	7	32	95,000	89,963	95%
	8	31	125,000	124,337	100%
PSA	1	32	37,500	36,110	96%
	2	32	375,000	372,237	99%
	3	32	1,500,000	1,309,999	87%
	4	32	150,000	171,612	114%
	5	32	7,500	9,025	120%
	6	32	15,000	18,199	121%
	7	31	3,000,000	2,554,682	85%
	8	31	2,280,000	2,198,033	96%

[1]Three samples (one sample in Panel Member 7 and two samples in Panel Member 8) had invalid PCA3 and/or PSA analyte results and were not included in the analyses.

From the clinical study, a total of 480 subjects out of 495 subjects that were eligible for analysis (97.0%) had a valid PCA3 Score: 4.0% (19/480) subjects had PCA3 analyte copies/mL in the range 250 copies/mL to 500 copies/mL and 1.3% (6/480) subjects had PSA analyte copies/mL in the range 7,500 copies/mL to 15,000 copies/mL. There were 0.8% (4/480) subjects having both PCA3 analyte and PSA analyte concentrations in these specified ranges; thus, there were 4.4% (21/480) unique subjects with specimen results for either or both analytes in the specified ranges.

Web Access to PROGENSA PCA3 Assay Labeling

The following labeling is available on the Gen-Probe website: www.gen-probe.com/package_msds/

- PROGENSA PCA3 Assay Package Insert
- Physician Brochure for the PROGENSA PCA3 Assay
- Physician Instructions for the PROGENSA PCA3 Assay

For a paper copy of any of these package inserts, please contact your sales representative or call:

+1 888 484 4747 or +1 858 410 8511

Or E-mail: technicalsupport@gen-probe.com

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Physician Brochure for the PROGENSA® PCA3 Assay

Exhibit 13

INTENDED USE

The PROGENSA PCA3 Assay is an *in vitro* nucleic acid amplification test. The assay measures the concentration of prostate cancer gene 3 (PCA3) and prostate-specific antigen (PSA) RNA molecules and calculates the ratio of PCA3 RNA molecules to PSA RNA molecules (PCA3 Score) in post-digital rectal exam (DRE) first-catch male urine specimens. The PROGENSA PCA3 Assay is indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PROGENSA PCA3 Assay results.

A PCA3 Score <25 is associated with a decreased likelihood of a positive biopsy. Prostatic biopsy is required for diagnosis of cancer.

WARNING:
The PROGENSA PCA3 Assay should not be used for men with atypical small acinar proliferation (ASAP) on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.

Warning: the Clinical Study only included men who were recommended by urologists for repeat biopsy. Therefore, the performance of the PROGENSA PCA3 Assay has not been established in men for whom a repeat biopsy was not already recommended.

LIMITATIONS OF THE CLINICAL STUDY RESULTS

- The PCA3 Score is intended to be used in conjunction with serum prostate-specific antigen (PSA) and other risk indicators to guide appropriate patient management in the "at risk" population of men who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended based on current standard of care.
- Performance of the PROGENSA PCA3 Assay has not been established in men who undergo repeat biopsy less than three months or more than seven years after their most recent negative biopsy.
- The effect of medications known to affect serum PSA levels such as finasteride (Proscar, Propecia), dutasteride (Avodart) and anti-androgen therapy (Lupron) on PROGENSA PCA3 Assay performance was not evaluated.
- Certain therapeutic and diagnostic procedures, such as prostatectomy, radiation, prostate biopsy and others, may affect the viability of prostatic tissue and subsequently impact the PCA3 Score. The effect of these procedures on assay performance has not yet been evaluated. Samples for PROGENSA PCA3 Assay testing should be collected when the clinician believes prostate tissue has recovered.
- Results from the PROGENSA PCA3 Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician and relevant guidelines in the decision for repeat biopsy.

TABLE OF CONTENTS

INTRODUCTION

The PROGENSA PCA3 Assay is an *in vitro* nucleic acid amplification test that measures the concentration of PCA3 RNA, a specific molecular marker for prostate cancer, and PSA RNA in male urine specimens. The assay result (called the "PCA3 Score") correlates with the likelihood of repeat biopsy outcome, meaning that a lower PCA3 Score is associated with a decreased likelihood of a positive repeat biopsy. The PCA3 Score is intended to be used in conjunction with serum prostate-specific antigen (PSA) and other risk indicators to guide appropriate patient management in the "at risk" population of men who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended based on current standard of care.

OVERVIEW

Prostate cancer (PCa) is the most common cancer and a leading cause of cancer death among men in the United States (American Cancer Society, 2011). Current practices for detecting PCa utilize serum PSA and DRE as indications for biopsy and approximately 25% of patients with elevated serum PSA are found to have PCa (Catalona, 1994; Schröder, 2009). This means that 75% of first biopsies are negative. The fear that cancer was missed often leads to repeat biopsies—most of which will also be negative because there is not enough clear information provided by serum PSA and DRE to decide whether to proceed with or delay an additional biopsy. Men with one or more previous negative biopsies present a clinical dilemma and there is a medical need for additional tests to help physicians and patients make more informed repeat biopsy decisions.

PCA3 is a prostate-specific gene that is highly over expressed in 95% of prostate cancers. Prostate cancer cells express 60 to 100 times more PCA3 RNA than normal cells (Hessels, 2003). The PROGENSA PCA3 Assay is highly-specific and uses Transcription Mediated Amplification (TMA) to quantify PCA3 RNA in a patient sample.

The PROGENSA PCA3 Assay is the first FDA-approved urine-based molecular test that detects the over-expression of the PCA3 gene. The specific information provided by the test (the PCA3 score) can be used in conjunction with other patient history to decide whether a repeat biopsy is necessary in men with one or more previous negative biopsies. Data on the PCA3 marker has been cited in over 100 publications (Salagierski, 2012) and more than 200,000 tests have been used in clinical practice throughout the world.

DEVICE DESCRIPTION

The PROGENSA PCA3 Assay utilizes whole urine collected following a digital rectal examination (DRE) consisting of three strokes per lobe. The DRE releases prostate cells through the prostate duct system into the urinary tract, where they can be collected in the first catch urine. The urine is processed by addition of Urine Transport Medium (UTM), which lyses the cells and stabilizes the RNA. PCA3 and PSA RNAs are quantified and the PCA3 Score is determined based on the ratio of PCA3 RNA to PSA RNA multiplied by 1000. In addition to normalizing PCA3 signal, measurement of PSA RNA also serves to confirm that the yield of prostate-specific RNA is sufficient to generate a valid result.

TECHNOLOGY

The PROGENSA PCA3 Assay is comprised of two quantitative nucleic acid amplification tests. The assay combines the technologies of target capture, TMA and Hybridization Protection Assay (HPA) to streamline urine specimen processing, amplify target RNA and detect amplicon, respectively.

When the PROGENSA PCA3 Assay is performed in the laboratory, the target RNA molecules are isolated from the urine specimens by target capture. Capture oligonucleotides that are complementary to sequence specific regions of the targets are hybridized to the targets in the urine specimen. A separate capture oligonucleotide is used for each target. The hybridized target is then captured onto magnetic microparticles that are separated from the urine specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube. Magnetic separation and wash steps are performed with a target capture system.

Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, Moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase. A unique set of primers is used for each target. The reverse transcriptase is used to generate a deoxyribonucleic acid (DNA) copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection is achieved by HPA using single-stranded, chemiluminescent-labeled nucleic acid probes that are complementary to the amplicon. Separate probes are used for each target amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The selection reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured in a luminometer and is reported as Relative Light Units (RLU).

PCA3 and PSA RNAs are quantified in separate tubes and the PCA3 Score is determined. Calibrators containing known amounts of PCA3 or PSA RNA transcript are included in every assay run and used to generate a standard curve. PCA3 and PSA controls are also included to verify the accuracy of results interpolated from the standard curve.

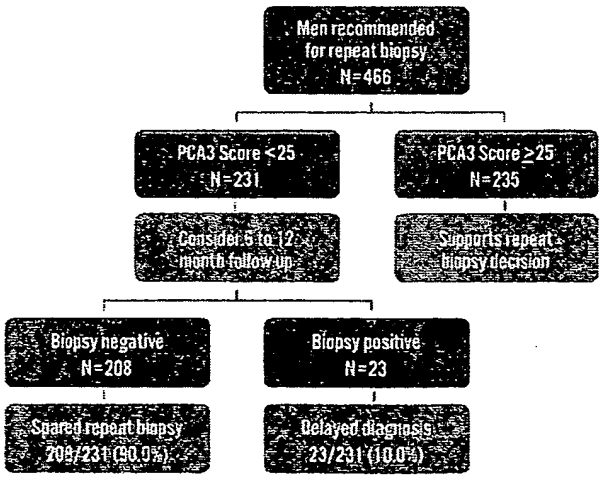
CLINICAL SENSITIVITY AND SPECIFICITY OF THE PROGENSA PCA3 ASSAY

A pivotal, prospective, multicenter clinical study was conducted to evaluate the performance of the PROGENSA PCA3 Assay for assessing the likelihood of repeat biopsy outcome. In order to evaluate the clinical performance of PROGENSA PCA3 Assay, the clinical study data were analyzed to determine how the use of the test might have affected the repeat biopsy recommendation for the clinical study subjects.

The flow chart below summarizes the results of this analysis. In the clinical study of 466 subjects, 49.6% (231 out of 466) of subjects had PCA3 Scores <25. This indicates decreased likelihood of a positive repeat biopsy result, so the clinician and patient might have considered delaying the repeat biopsy. Of these 231 men, 208 (90%) subsequently had a negative biopsy result, while 23 (10%) had a biopsy positive for prostate cancer. For the 235 men with PCA3 Scores ≥25, the PROGENSA PCA3 Assay result supports the decision to repeat biopsy (34% (79/235) of these men had positive biopsies).

The potential clinical benefit is that 44.6% (208 out of 466) of men in the study may have been spared an unnecessary repeat biopsy. Instead, these men would have been monitored closely for any change in risk factors that would suggest disease. The associated risk is that 23 of the men who had a biopsy positive for prostate cancer may have had their diagnosis delayed. The standard timeframe for conducting follow up with the intended use population is 6 to 12 months. In terms of risk versus benefit, nine men may have avoided an unnecessary repeat biopsy for every one man whose diagnosis may be delayed. In the context of the entire study population (466 total subjects), 49.6% (231/466) of prostate biopsies would have been avoided and 4.9% (23/466) of men who harbored biopsy-detectable prostate cancer would have been monitored instead of receiving an immediate repeat biopsy. The PROGENSA PCA3 Assay should not be used for men with ASAP on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.

SUMMARY OF CLINICAL STUDY RESULTS



CLINICAL STUDY RESULTS

Four hundred ninety-five (495) male subjects were enrolled from a total of 14 clinical sites, including academic institutions, community-based urology clinics and group health organizations. Men who had at least one previous negative prostatic biopsy, who had never had a positive prostatic biopsy, and who had been recommended for a repeat biopsy by their urologists were eligible for study participation. A questionnaire was used to collect information regarding prostate cancer risk factors from men recommended for a repeat biopsy by their clinician (i.e., the enrolled population) and from men not recommended for a repeat biopsy (i.e., the non-enrolled population). Age, prostate volume and most recent free PSA test result were not significantly different between enrolled and non-enrolled populations. Serum PSA test results and the time since the most recent negative biopsy were significantly different (P<.0001) between enrolled and non-enrolled populations, where the non-enrolled men (men not recommended for repeat biopsy by their clinician) had 2.2 ng/mL lower mean serum PSA test results and approximately 60% shorter time since their most recent previous negative biopsy. Clinical study analysis included men who had been recommended for a repeat biopsy by their urologists.

Blood, urine and prostatic biopsy specimens were collected from each subject. The blood specimen was tested with a total serum PSA test at the collection site's associated testing facility. The total serum PSA test used varied by collection site. The urine specimen was collected following a digital rectal exam (DRE) and was a first-catch urine specimen. The urine specimen was processed at the collection site by aliquotting into PROGENSA PCA3 Urine Specimen Transport Tubes and shipped to a testing site for PROGENSA PCA3 Assay testing. The prostatic biopsy was performed per the collection site's standard procedure. The biopsy specimens were evaluated by the collection site's associated pathology facility(ies).

For the 495 eligible subjects, the median age was 67.0 years; ages ranged from 44 years to 92 years. Race was reported as White for 433 subjects (87.5%), Black or African American for 45 subjects (9.1%), Asian for 11 subjects (2.2%), American Indian/Alaska Native for 2 subjects (0.4%) and unknown for 5 subjects (1.0%). Four hundred eighty (480) of the eligible subjects provided a urine sample for PROGENSA PCA3 Assay testing (3.0% (15/495) of subjects did not provide a urine sample); 1.3% (6/480) of sample results were excluded because of sample qualification failure (insufficient RNA for accurate analysis), leaving 474 subjects with a valid and reportable PCA3 Score.

Four hundred sixty-six (466) subjects with valid and reportable PCA3 Scores and disease status (determined by biopsy result) and who were 50 years of age or older were included in the analyses. Prevalence of positive repeat biopsy was 21.9% (102/466). For the subjects with a study total serum PSA test result (n=464), the

median total serum PSA test result was 5.80 ng/mL (results ranged from 0.3 ng/mL to 49.2 ng/mL). Prostatic biopsies consisted of 6 to 24 cores with 93% of subjects having 12 to 21 cores taken.

Figure 1. Shows the percentage of subjects with positive prostatic biopsy results by PCA3 Score interval (with 95% confidence).

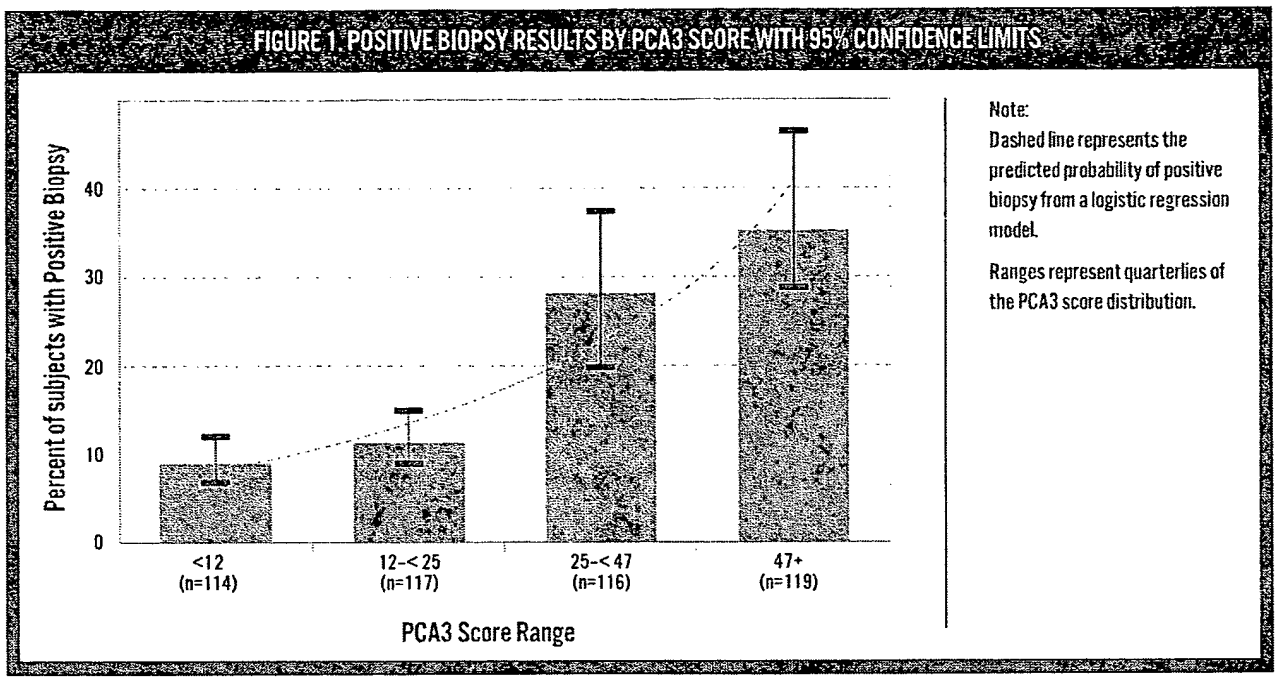


Table 1. Shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome at a PCA3 Score cut-off value of 25.

TABLE 1. PERFORMANCE CHARACTERISTICS OF THE PROGENSA PCA3 ASSAY

	BIOPSY POSITIVE	BIOPSY NEGATIVE	TOTAL
PCA3 SCORE ≥ 25	79	156	235
PCA3 SCORE < 25	23	208	231
TOTAL	102	364	466
POSITIVE BIOPSY PREVALENCE %	21.9 (102/466)		

PERFORMANCE CHARACTERISTICS	ESTIMATE	95% CI
SENSITIVITY %	77.5 (79/102)	68.4-84.5
SPECIFICITY %	57.1 (208/364)	52.0-62.1
PPV %	33.6 (79/235)	30.0-37.2
NPV %	90.0 (208/231)	86.5-93.1
PLR	1.81	1.53-2.11
NLR	0.40	0.26-0.56
ODDS RATIO	4.58	2.75-7.62

CI = Confidence Interval, PPV = Positive Predictive Value, NPV = Negative Predictive Value, PLR = Positive Likelihood Ratio, NLR = Negative Likelihood Ratio

PROGENSA PCA3 ASSAY PERFORMANCE IN SUBGROUPS

Table 2. Shows the performance characteristics of the PROGENSA PCA3 Assay relative to prostatic biopsy outcome for subgroups of the study population.

Table 3. Shows PROGENSA PCA3 Assay performance results in the subgroup of men with ASAP on their most recent negative biopsy. The clinical study was not designed to evaluate subgroups, so the results for individual subgroups may not be conclusive. However, PROGENSA PCA3 Assay performance in men with prior ASAP indicated that the PROGENSA PCA3 Assay is not informative of biopsy outcome in this subgroup (Table 3).

TABLE 2: PERFORMANCE CHARACTERISTICS OF THE PROGENSA PCA3 ASSAY BY SUBGROUPS

SUBGROUP	N	TP	FP	TN	FN	SE% (95% CI)[1]	SP% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	PLR (95% CI)	NLR (95% CI)	OR (95% CI)
AGE (YEARS)												
50-59	95	10	19	59	8	55.6 (33.7-75.4)	75.6 (65.1-83.8)	34.5 (20.8-48.0)	88.1 (82.2-93.5)	2.28 (1.14-4.00)	0.59 (0.30-0.94)	3.88 (1.34-11.25)
60-69	193	24	62	94	13	64.9 (48.8-78.2)	60.3 (52.4-67.6)	27.9 (21.4-34.2)	87.9 (82.7-92.5)	1.63 (1.15-2.19)	0.58 (0.34-0.88)	2.8 (1.33-5.91)
70+	177	45	75	55	2	95.7 (85.8-98.8)	42.3 (34.2-50.9)	37.5 (33.6-41.6)	96.5 (89.5-99.5)	1.66 (1.40-1.97)	0.10 (0.02-0.33)	16.50 (3.84-70.94)
PRIOR NEGATIVE BIOPSY RESULT												
HGPIN (NOT ASAP)	101	21	42	35	3	87.5 (69.0-95.7)	45.5 (34.8-56.5)	33.3 (27.1-39.5)	92.1 (81.7-98.2)	1.60 (1.20-2.09)	0.28 (0.06-0.72)	5.83 (1.61-21.20)
NONE/OTHER	316	48	90	163	15	76.2 (64.4-85.0)	64.4 (58.4-70.1)	34.8 (29.8-39.9)	91.6 (87.8-94.7)	2.14 (1.71-2.65)	0.37 (0.22-0.56)	5.80 (3.07-10.93)
NUMBER OF PREVIOUS NEGATIVE BIOPSIES												
1	316	56	101	138	21	72.7 (61.9-81.4)	57.7 (51.4-63.8)	35.7 (31.0-40.4)	86.8 (82.2-90.9)	1.72 (1.39-2.10)	0.47 (0.31-0.67)	3.64 (2.07-6.40)
2+	150	23	55	70	2	92.0 (75.0-97.8)	56.0 (47.2-64.4)	29.5 (24.3-34.6)	97.2 (91.9-99.6)	2.09 (1.61-2.65)	0.14 (0.02-0.44)	14.64 (3.31-64.78)
TIMING OF PREVIOUS BIOPSY RELATIVE TO STUDY ENROLLMENT												
<3 MONTHS[1]	13	2	6	2	3	40.0 (11.8-76.9)	25.0 (7.1-59.1)	25.0 (3.2-49.9)	40.0 (6.9-76.2)	0.53 (0.05-1.59)	2.40 (0.50-21.76)	0.22 (0.02-2.45)
3 MONTHS -<7 YEARS	438	75	145	199	19	79.8 (70.6-86.7)	57.8 (52.6-63.0)	34.1 (30.4-37.8)	91.3 (87.7-94.3)	1.89 (1.60-2.22)	0.35 (0.22-0.51)	5.42 (3.13-9.36)
7+ YEARS	15	2	5	7	1	66.7 (20.8-93.9)	58.3 (32.0-80.7)	28.6 (6.5-54.8)	87.5 (66.6-99.5)	1.60 (0.28-4.85)	0.57 (0.02-2.01)	2.80 (0.20-40.06)
RACE												
BLACK	39	6	16	16	1	85.7 (48.7-97.4)	50.0 (33.6-66.4)	27.3 (14.3-37.9)	94.1 (79.4-99.8)	1.71 (0.76-2.79)	0.29 (0.01-1.19)	6.00 (0.65-55.66)
NON-BLACK	427	73	140	192	22	76.8 (67.4-84.2)	57.8 (52.5-63.0)	34.3 (30.5-38.1)	89.7 (86.0-92.9)	1.82 (1.53-2.15)	0.40 (0.27-0.57)	4.55 (2.69-7.69)
SERUM PSA (NG/ML) AND DIGITAL RECTAL EXAM												
PSA<4 AND DRE NORM	81	13	37	28	3	81.3 (57.0-93.4)	43.1 (31.6-55.2)	26.0 (18.8-32.3)	90.3 (78.5-97.6)	1.43 (0.94-1.94)	0.44 (0.10-1.11)	3.28 (0.85-12.62)
PSA≥4 OR DRE ABN	383	65	119	179	20	76.5 (66.4-84.2)	60.1 (54.4-65.5)	35.3 (31.1-39.6)	89.9 (86.1-93.3)	1.92 (1.58-2.30)	0.39 (0.25-0.56)	4.89 (2.81-8.49)
SERUM PSA (NG/ML) AND NUMBER OF PREVIOUS NEGATIVE BIOPSIES												
PSA>10 AND 1 BX	34	10	9	14	1	90.9 (62.3-98.4)	60.9 (40.8-77.8)	52.6 (38.4-68.4)	93.3 (73.4-99.8)	2.32 (1.31-4.53)	0.15 (0.01-0.78)	15.56 (1.69-143.16)
PSA≤10 OR 2+ BX	430	68	147	193	22	75.6 (65.8-83.3)	56.8 (51.5-61.9)	31.6 (27.9-35.3)	89.8 (86.1-93.0)	1.75 (1.46-2.06)	0.43 (0.29-0.61)	4.06 (2.39-6.87)

TP = true positive, FP = false positive, TN = true negative, FN = false negative, CI = confidence interval, SE = sensitivity, SP = specificity, PPV (NPV) = positive (negative) predictive value, PLR (NLR) = positive (negative) likelihood ratio, OR = odds ratio, Bx = biopsy. For calculations in this table, PCA3 Score values ≥ 25 are considered positive and PCA3 score values < 25 are considered negative.

[1] In this subgroup, 84.6% (11/13) had ASAP on their most recent negative biopsy. In the clinical study, the PROGENSA PCA3 Assay was not predictive of repeat biopsy outcome in men with prior ASAP (Table 3).

TABLE 3. PERFORMANCE CHARACTERISTICS OF PROGENSA PCA3 ASSAY IN MEN WITH ASAP ON THEIR MOST RECENT NEGATIVE BIOPSY

	BIOPSY POSITIVE	BIOPSY NEGATIVE	TOTAL
PCA3 SCORE ≥ 25	10	24	34
PCA3 SCORE < 25	5	10	15
TOTAL	15	34	49
POSITIVE BIOPSY PREVALENCE %	30.6 (15/49)		

PERFORMANCE CHARACTERISTICS	ESTIMATE	95% CI
SENSITIVITY %	66.7 (10/15)	41.7-84.8
SPECIFICITY %	29.4 (10/34)	16.8-46.2
PPV %	29.4 (10/34)	19.1-38.2
NPV %	66.7 (10/15)	44.7-87.0
PLR	0.94	0.54-1.40
NLR	1.13	0.34-2.80
ODDS RATIO	0.83	0.23-3.07

CI = confidence interval, PPV = positive predictive value, NPV = negative predictive value, PLR = positive likelihood ratio, NLR= negative likelihood ratio

*The PROGENSA PCA3 Assay should not be used for men with ASAP on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.

THE ADDITION OF PROGENSA PCA3 ASSAY INFORMATION IMPROVES DIAGNOSTIC ACCURACY OVER EXISTING STANDARD OF CARE FACTORS

Multivariable logistic regression analysis was conducted to determine whether the addition of the PROGENSA PCA3 Assay information improved diagnostic accuracy over the standard of care information that is currently used for repeat biopsy decisions. The standard of care factors included the following: age, DRE result, family history, race, serum PSA test result and number of previous negative biopsies.

Table 4. Shows the results from the multivariable logistic regression analysis. In this analysis, the odds ratio (OR) for PCA3 Score (expressed as a binary categorical variable [positive or negative using a cutoff of 25]) was statistically significant (P< .0001). These results indicate that the PCA3 Score is a statistically significant predictor of repeat biopsy outcome in the presence of current standard of care factors used in the decision to perform a repeat biopsy.

TABLE 4. MULTIVARIABLE LOGISTIC REGRESSION RESULTS FOR THE OCCURRENCE OF PROSTATE CANCER ASSOCIATED WITH PCA3 SCORE USING A BINARY CUTOFF OF 25 AND OTHER CLINICAL FACTORS

FACTOR*	REGRESSION COEFFICIENT (SE)	ODDS RATIO (95% CI)	P-VALUE
PCA3 SCORE (≥ 25 VS. <25)	1.5175 (0.2762)	4.5610 (2.6542, 7.8376)	<.0001
AGE IN YEARS (CONTINUOUS)	0.0073 (0.0158)	1.0073 (0.9766, 1.0389)	.6458
SUSPICIOUS DRE (YES VS. NO)	0.0251 (0.2801)	1.0254 (0.5928, 1.7753)	.9287
FAMILY HISTORY (ANY VS. NONE)	-0.0795 (0.3162)	0.9235 (0.4970, 1.7163)	.8014
FAMILY HISTORY (UNKNOWN/REFUSED VS. NONE)	0.3756 (0.5054)	1.4558 (0.5406, 3.9203)	.4574
RACE (BLACK VS. NON-BLACK)	0.5506 (0.4700)	0.5766 (0.2295, 1.4485)	.2414
SERUM PSA IN NG/ML (CONTINUOUS)	0.0669 (0.0215)	1.0691 (1.0250, 1.1152)	.0019
NUMBER OF PREVIOUS NEGATIVE BIOPSIES (2 VS. 1)	-0.7955 (0.3259)	0.4513 (0.2383, 0.8549)	.0146
NUMBER OF PREVIOUS NEGATIVE BIOPSIES (3+ VS. 1)	-0.8028 (0.4545)	0.4481 (0.1839, 1.0921)	.0774

SE = standard error, CI = confidence interval

Note: A total of N=464 subjects from the Full Analysis Set have complete data for all of the factors in the multivariable logistic regression analysis.

* Per the statistical analysis plan, prostate volume (continuous) was not included as a standard of care covariate, as the regression coefficient associated with prostate volume was not statistically significant at a .05 level (P= .0583) and the regression coefficient for PCA3 Score changed by less than 10% when prostate volume was removed from the model (actual observation 1.5%).

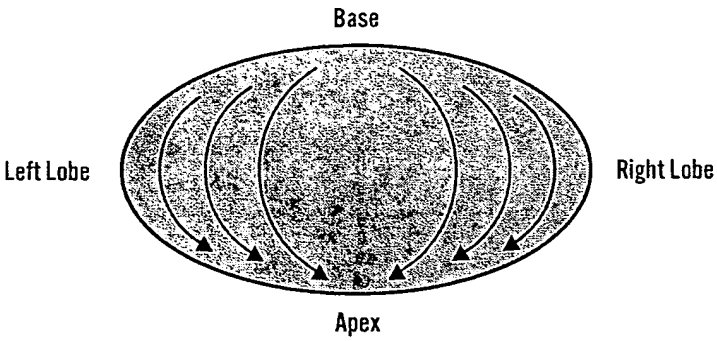
The PCA3 Score was also evaluated as a continuous measure. The OR for PCA3 Score (continuous) was 1.0099 (95% CI: 1.0058 – 1.0140; P < .0001). This indicates that each PCA3 Score unit increase represents a 0.99% increase in the likelihood of a positive repeat biopsy. Thus, PCA3 Score is also a statistically significant and independent predictor of the likelihood of positive repeat biopsy when measured incrementally.

SPECIMEN COLLECTION– IMPORTANT CONSIDERATIONS

Sample Preparation (Instructions For Urine Specimen Collection and Processing):

1. Conduct a DRE as described below immediately prior to urine collection: Apply pressure on the prostate, enough to depress the surface, from the base to the apex and from the lateral to the median line for each lobe as shown in Figure 2. Perform exactly three strokes for each lobe. This is NOT intended to be a prostatic massage.

Figure 2. Proper Direction of Applied Prostrate Pressure



2. Following the DRE, direct the patient to provide first-catch urine (approximately 20 to 30 mL of the initial urine stream) in an appropriately labeled urine collection cup. This must be the first voided urine specimen following the DRE. Use a collection cup free of any preservatives. If a patient cannot stop his urine flow and provides more urine than the requested first 20 to 30 mL, keep the entire volume. Very high urine volumes can lower PCA3 and PSA analyte concentrations and may infrequently result in an invalid specimen. Thus, the patient should try to avoid filling the urine collection cup. If the patient is unable to provide the requested volume of urine (at least 2.5 mL is required to run the PROGENSA PCA3 Assay) then the specimen must be rejected.

3. **Unprocessed urine specimens, if not immediately processed, must be maintained at 2°C to 8°C or kept on ice. The chilled, unprocessed urine specimen must be transferred into the urine specimen transport tube within four hours of collection. Otherwise, the specimen must be rejected and the urologist must collect a new specimen. Do not freeze unprocessed urine specimens.**

4. To process urine specimens, tightly cap and invert the urine specimen five times to resuspend cells. Remove the cap of the urine specimen transport tube and transfer 2.5 mL of the collected urine into the tube using the disposable transfer pipette provided. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine specimen transport tube label.

5. Re-cap the urine specimen transport tube tightly and invert the urine specimen five times to mix. This is now known as the processed urine specimen.

6. Avoid cross-contamination during the specimen handling steps. Urine specimens can contain high levels of RNA target. Ensure specimen containers do not contact each other and discard used materials without passing them over any containers. If gloves come in contact with a specimen, change gloves to avoid cross-contamination.

7. Ship or transport processed urine specimens according to instructions provided by your testing laboratory. Shipping arrangements must be made to ensure specimens are received by the testing site within five days of collection.

PROCESSED SAMPLE TRANSPORT AND STORAGE BEFORE TESTING:

1. Processed urine specimens must be transported to the laboratory in the urine specimen transport tube. They may be shipped under ambient conditions (without temperature control) or frozen. Shipping arrangements must be made to ensure specimens are received by the testing site within five days of collection. Upon receipt of the shipment, the laboratory should verify the date of specimen collection on the tube. If specimens were shipped under ambient conditions and are received greater than five days after specimen collection, the specimen must be rejected and a request for a new specimen should be made. The laboratory may store specimens at 2°C to 8°C for up to 14 days before disposal is required. If longer time periods are needed, refer to Table 6 for the allowable storage times at different temperatures.

TABLE 6. PROCESSED URINE SPECIMEN STORAGE DURATIONS

STORAGE TEMPERATURE	TIME
PROCESSED SPECIMEN STORAGE AND SHIPMENT:	
AT OR BELOW 30°C	UP TO 5 DAYS*
AFTER RECEIPT AT TESTING SITE:	
2°C TO 8°C	UP TO 14 DAYS
-35°C TO -15°C	UP TO 11 MONTHS**
AT OR BELOW -65°C	UP TO 36 MONTHS**

*Time allowed for shipment under ambient conditions or frozen.
**Time allowed after refrigerated storage.

2. Processed urine specimens may be subjected to up to five freeze–thaw cycles.

INTERPRETATION OF PROGENSA PCA3 ASSAY RESULTS

The PCA3 Score is calculated as the ratio of PCA3 RNA copies to PSA RNA copies, multiplied by 1000. As the PCA3 Score increases, the likelihood for a positive biopsy increases. As the PCA3 Score decreases, the likelihood for a positive biopsy decreases.

If the reported PCA3 Score is below the cut-off of 25, the result should be interpreted as NEGATIVE. If the PCA3 Score is above or equal to the cut-off of 25, the result should be interpreted as POSITIVE. A NEGATIVE result is associated with decreased likelihood of a positive biopsy.

Due to normal assay variability, specimens with PCA3 Scores near the cutoff of 25 (i.e., 18 to 31) could yield a different overall interpretation of POSITIVE or NEGATIVE upon repeat testing. PCA3 Scores in the range from 18 to 31 should therefore be interpreted with caution. The PCA3 Score should be used in conjunction with other patient information to aid in the decision for repeat biopsy.

Sometimes a <[Calculated Score] or >[Calculated Score] is reported. This occurs if the PCA3 and/or PSA analyte concentration is outside the quantitative range. If < [Calculated Score] is below the cut-off of 25, the result should be interpreted as NEGATIVE. If >[Calculated Score] is above the cut-off of 25, the result should be interpreted as POSITIVE. In some cases, it may not be possible to determine if a specimen is POSITIVE or NEGATIVE. If the PCA3 Score is indeterminate relative to the cutoff of 25, another specimen must be collected from the patient and retested.

The laboratory may request an additional urine specimen if the laboratory cannot provide a PCA3 Score that can be used for interpretation relative to the cutoff of 25.

Footnotes

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GEN-PROBE

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Exhibit 14

Alan Maderazo, Ph.D., RAC
Associate Director, Regulatory Affairs
Gen-Probe Incorporated
10210 Genetic Center Drive
San Diego, CA 92121

FEB 13 2012

Re: P100033
PROGENSA® PCA3 assay
Filed: August 10, 2010
Amended: October 25, 2010, November 10, 2010, January 25, 2011, May 16, 2011, August 12, 2011, October 11, 2011, January 27, 2012, February 9, 2012
Procode: OYM

Dear Dr. Maderazo:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the PROGENSA® PCA3 Assay. This device is indicated for:

The PROGENSA PCA3 Assay is an in vitro nucleic acid amplification test. The assay measures the concentration of prostate cancer gene 3 (PCA3) and prostate-specific antigen (PSA) RNA (RNA) molecules and calculates the ratio of PCA3 RNA molecules to PSA RNA molecules (PCA3 Score) in post digital rectal exam (DRE) first catch male urine specimens. The PROGENSA PCA3 Assay is indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PROGENSA PCA3 Assay results.

A PCA3 Score <25 is associated with a decreased likelihood of a positive biopsy. Prostatic biopsy is required for diagnosis of cancer.

We are pleased to inform you that the PMA is approved. You may begin commercial distribution of the device in accordance with the conditions of approval described below.

The sale and distribution of this device are restricted to prescription use in accordance with 21 CFR 801.109 and under section 515(d)(1)(B)(ii) of the Federal Food, Drug, and Cosmetic Act (the act). The device is further restricted under section 515(d)(1)(B)(ii) of the act insofar as the labeling must specify the specific training or experience practitioners need in order to use the device. FDA has determined that these restrictions on sale and distribution are necessary to provide reasonable assurance of the safety and effectiveness of the device. Your device is therefore a restricted device

subject to the requirements in sections 502(q) and (r) of the act, in addition to the many other FDA requirements governing the manufacture, distribution, and marketing of devices.

Expiration dating for this device has been established and approved at (a) 18 months for the Amplification Reagents (PCA3 and PSA), Probe Reagents (PCA3 and PSA), PCA3/PSA Enzyme Reagent, Calibrators (PCA3 and PSA), and Controls (PCA3 and PSA), when stored at 2-8°C, (b) 18 months for the PCA3/PSA Amplification Reconstitution Solution, PCA3/PSA Probe Reconstitution Solution, PCA3/PSA Enzyme Reconstitution Solution, and PCA3/PSA Selection Reagent, when stored at 2-30°C, (c) 16 months for the Target Capture Reagents (PCA3 and PSA), when stored at 15-30°C, (d) 24 months for the APTIMA Assay Fluids (Oil Reagent, Wash Solution and Buffer for Deactivation Fluid), when stored at 15-30°C, and (e) 20 months for the Urine transportation kit, when stored at 28°±2°C. This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(7).

Continued approval of this PMA is contingent upon the submission of periodic reports, required under 21 CFR 814.84, at intervals of one year (unless otherwise specified) from the date of approval of the original PMA. Two copies of this report, identified as "Annual Report" (please use this title even if the specified interval is more frequent than one year) and bearing the applicable PMA reference number, should be submitted to the address below. The Annual Report should indicate the beginning and ending date of the period covered by the report and should include the information required by 21 CFR 814.84.

In addition to the above, and in order to provide continued reasonable assurance of the safety and effectiveness of the device, the Annual Report must include, separately for each model number (if applicable), the number of devices sold and distributed during the reporting period, including those distributed to distributors. The distribution data will serve as a denominator and provide necessary context for FDA to ascertain the frequency and prevalence of adverse events, as FDA evaluates the continued safety and effectiveness of the device.

You have agreed that all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a statement of the intended uses of the device and relevant warnings shown below.

> Black Box Warning: The PROGENSA PCA3 Assay should not be used for men with atypical small acinar proliferation (ASAP) on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.
>
> Warning: The Clinical Study only included men who were recommended by urologists for repeat biopsy. Therefore, the performance of the PROGENSA PCA3 Assay has not been established in men for whom a repeat biopsy was not already recommended.

Before making any change affecting the safety or effectiveness of the device, you must submit a PMA supplement or an alternate submission (30-day notice) in accordance with 21 CFR 814.39. All PMA supplements and alternate submissions (30-day notice) must comply with the applicable

requirements in 21 CFR 814.39. For more information, please refer to the FDA guidance document entitled, "Modifications to Devices Subject to Premarket Approval (PMA) - The PMA Supplement Decision-Making Process" (www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089274.htm).

You are reminded that many FDA requirements govern the manufacture, distribution, and marketing of devices. For example, in accordance with the Medical Device Reporting (MDR) regulation, 21 CFR 803.50 and 21 CFR 803.52, you are required to report adverse events for this device. Manufacturers of medical devices, including in vitro diagnostic devices, are required to report to FDA no later than 30 calendar days after the day they receive or otherwise becomes aware of information, from any source, that reasonably suggests that one of their marketed devices:

1. May have caused or contributed to a death or serious injury; or

2. Has malfunctioned and such device or similar device marketed by the manufacturer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

Additional information on MDR, including how, when, and where to report, is available at www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

In accordance with the recall requirements specified in 21 CFR 806.10, you are required to submit a written report to FDA of any correction or removal of this device initiated by you to: (1) reduce a risk to health posed by the device; or (2) remedy a violation of the act caused by the device which may present a risk to health, with certain exceptions specified in 21 CFR 806.10(a)(2). Additional information on recalls is available at www.fda.gov/Safety/Recalls/IndustryGuidance/default.htm.

CDRH does not evaluate information related to contract liability warranties. We remind you; however, that device labeling must be truthful and not misleading. CDRH will notify the public of its decision to approve your PMA by making available, among other information, a summary of the safety and effectiveness data upon which the approval is based. The information can be found on the FDA CDRH Internet HomePage located at www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/PMAApprovals/default.htm. Written requests for this information can also be made to the Food and Drug Administration, Dockets Management Branch, (HFA-305), 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. The written request should include the PMA number or docket number. Within 30 days from the date that this information is placed on the Internet, any interested person may seek review of this decision by submitting a petition for review under section 515(g) of the act and requesting either a hearing or review by an independent advisory committee. FDA may, for good cause, extend this 30-day filing period.

Failure to comply with any post-approval requirement constitutes a ground for withdrawal of approval of a PMA. The introduction or delivery for introduction into interstate commerce of a device that is not in compliance with its conditions of approval is a violation of law.

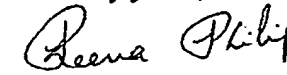
You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form. Final printed labeling that is identical to the labeling approved in draft form will not routinely be reviewed by FDA staff when accompanied by a cover letter stating that the final printed labeling is identical to the labeling approved in draft form. If the final printed labeling is not identical, any changes from the final draft labeling should be highlighted and explained in the amendment.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing. One of those three copies may be an electronic copy (eCopy), in an electronic format that FDA can process, review and archive (general information: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/ucm134508.htm; clinical and statistical data: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/ucm136377.htm)

U.S. Food and Drug Administration
Center for Devices and Radiological Health
PMA Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

If you have any questions concerning this approval order, please contact Nisar Pampori at 301-796-6144.

Sincerely yours,



For Maria M. Chan., Ph.D.
Director, Division of Immunology and Hematology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Exhibit 15

Chesapeake Research Review, Inc.

Providing Human Research Protections Services
IRB Services • Consultation • Education
7063 Columbia Gateway Drive, Suite 110
Columbia, MD 21046-3403
410.884.2900

PROTOCOL APPROVAL

DATE: 31 Jul 2009

TO: Meenal Patel
Clinical Trial Manager, Gen-Probe, Inc

FROM: April Bost, CCRC
Project Coordinator, Chesapeake IRB

RE: Initial Review of Gen-Probe Protocol 2009PCA301-Enroll, Clinical Evaluation of the PROGENSA™ PCA3 Assay in Men With a Previous Negative Biopsy Result – Enrollment and Specimen Collection (Pro00003247)

Thank you for selecting Chesapeake IRB to provide oversight for your research project. The IRB has reviewed the following:

- Protocol 2009PCA301-Enroll (Dated July 8, 2009).
- PCA3 IDE Letter (Dated July 17, 2009).
- PCA3 Non Invasive Device Letter (Dated July 17, 2009).
- Informed Consent and Authorization Form.
- Device Information for PROGENSA® PCA3 (501826 Rev1, Dated 2009-05).
- PCA3 SUBJECT SCREENING QUESTIONNAIRE

The IRB approved the above referenced protocol on 24 Jul 2009. The IRB Approved Informed Consent and Authorization form template (CRRI v.072909) is electronically available on your workspace.

Please Note: Each Principal Investigator will receive a separate IRB Approval notice allowing them to conduct the study.

IRB Approval for the protocol expires on 24 Jul 2010 unless re-approved by the IRB. A Continuing Review reminder will be sent prior to your expiration date.

Please review the Terms of IRB Oversight by accessing CIRBI™ (www.cirbi.net). Log on to your homepage ("My Home") and select the "Reference Materials" tab for IRB requirements and guidance. A copy of the most recent IRB roster is also available under "Reference Materials".

If you have any questions or concerns, please use the Contact IRB activity on your workspace. We look forward to continuing to work with you on this project.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Exhibit 16

Food and Drug Administration
10903 New Hampshire Avenue
Document Mail Center – WO66-0609
Silver Spring, MD 20993-0002

RECEIVED
SEP 17 2010
Mkt. Reg. Affairs

Alan Maderazo, Ph.D., RAC
Associate Director, Regulatory Affairs
Gen-Probe Incorporated
10210 Genetic center Drive
San Diego, CA 92121-4362

SEP 8 2010

Re: P100033
PROGENSA® PCA3 Assay
Filed: August 10, 2010

Dear Dr. Maderazo:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed an initial review of your premarket approval application (PMA) supplement. We are pleased to inform you that we have made a threshold determination that the PMA supplement is sufficiently complete to permit a substantive review and is, therefore, suitable for filing. The filing date is August 10, 2010, which is the date of CDRH receipt of the PMA supplement.

Please be advised that the decision to file the PMA does not imply that either an in-depth evaluation of the safety and effectiveness of the device has been performed or a decision about the approvability of the application has been made. Rather, it represents a decision by CDRH that the application is sufficiently complete to begin the substantive review process. Further review of your application may result in deficiencies which will be communicated to you.

Following receipt of a filing letter, an applicant is required by 21 CFR 814.20(e) to update its pending PMA supplement 3 months after the filing date with new safety and effectiveness information learned about the device from ongoing or completed studies when the information may reasonably affect an evaluation of the safety or effectiveness of the device or that may reasonably affect the statement of contraindications, warnings, precautions and adverse reactions in the draft labeling.

This updated reporting is limited to studies sponsored by the applicant or to which the applicant has reasonable access. The update report should be consistent with the data reporting provisions of the protocol. Please submit clinical updates in three copies as an amendment to the PMA supplement and include the FDA reference number assigned to the PMA supplement.

The PMA cannot be approved until FDA has determined that the manufacturing facilities, methods and controls comply with the conditions set forth in your application and the applicable

requirements of the Quality System Regulation (21 CFR Part 820). If you have not already done so, please notify CDRH as soon as possible in the form of an amendment to the PMA if there will be a delay in setting up your manufacturing facility for production of the device, and provide the expected date that the facility will be prepared for an FDA inspection. If you have any questions regarding the status of your Quality System inspection please contact the Office of Compliance at (301) 796-5815, or your District Office.

A meeting of the Immunology Panel will be held at which your PMA supplement will be reviewed. You will be notified of the location and date of this meeting. Any additional information to be included in your PMA supplement should be submitted in the form of a PMA supplement amendment and be received by FDA at least 8 weeks in advance of the scheduled advisory panel meeting in order for FDA and the panel members to have adequate time to review the new information. Information received by CDRH less than 8 weeks in advance of a scheduled advisory panel meeting will not be considered or reviewed at the meeting and may delay consideration of your PMA supplement until a subsequent advisory panel meeting.

For your information, there is an industry representative on this FDA advisory panel whose name, address and telephone number you can obtain by contacting the Committee Management Staff at (301) 796-5964. CDRH believes that industry representatives will be better prepared to participate in panel discussions if they have been provided with at least a copy of the Summary of Safety and Effectiveness Data for review prior to the panel meeting. In accordance with 21 CFR 14.86(b), all panel members are subject to all rules and regulations adopted by FDA and the committee; therefore, even though the industry representatives usually are not given access to trade secret and confidential, commercial information, they are bound to protect the confidentiality of documents that would be sent to them in preparation for panel review of a PMA. If you would like the industry representative to have access to any portion of your PMA, including the Summary of Safety and Effectiveness Data, please provide a copy to FDA for that purpose. Clearly identify the submission as a purged copy intended for review by the industry representative. Review of your PMA will not be prejudiced if you elect not to provide information for industry representative review.

All correspondence regarding this PMA supplement should be submitted in six (6) copies in the form of a PMA supplement amendment. Please address all submissions to:

U.S. Food and Drug Administration
Center for Devices and Radiological Health
PMA Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

If you have any questions regarding this letter, please contact Dai J. Li, MD, Ph.D. at 301-796-6174.

Sincerely yours,

Maria M. Chan

Maria M. Chan, Ph.D.
Director
Division of Hematology and Immunology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

PROGENSA® PCA3 Assay
PMA P100033
Pre-IDE # I060180 / I090310
Reverse Chronological Correspondence Index

Exhibit 17

DATE ORIG	DATE REC'D	TO	FROM	REFERENCE
05/06/10				Teleconference - Gen-Probe and FDA
04/06/10		FDA	Gen-Probe	Sent document
03/29/10		Gen-Probe	FDA	Sent document
03/26/10		FDA	Gen-Probe	Request
03/13/10		Gen-Probe	FDA	Instructions
03/12/10		FDA	Gen-Probe	Request
03/12/10		FDA	Gen-Probe	Request
02/22/10		Gen-Probe	FDA	Sent document
02/03/10				Teleconference – Gen-Probe and FDA and FDA
01/29/10		FDA	Gen-Probe	Sent document
01/28/10		Gen-Probe	FDA	Scheduling
01/27/10		FDA	Gen-Probe	Request
01/12/10		FDA	Gen-Probe	Confirmation
01/12/10		Gen-Probe	FDA	Sent document
01/11/10		FDA	Gen-Probe	Email confirmation
01/11/10		Gen-Probe	FDA	Email
01/11/10		FDA	Gen-Probe	Telephone call
01/11/10		Gen-Probe	FDA	Telephone call
01/08/10		FDA	Gen-Probe	Telephone call
01/07/10		FDA	Gen-Probe	Telephone call and email
01/04/10		Gen-Probe	FDA	Follow-up
01/04/10		FDA	Gen-Probe	Telephone call
12/29/09		FDA	Gen-Probe	Telephone call
12/18/09		FDA	Gen-Probe	Email
11/24/09		FDA	Gen-Probe	Email
10/30/09		FDA	Gen-Probe	Email
10/19/09				Face to face meeting – FDA and Gen-Probe
09/29/09		FDA	Gen-Probe	Email
08/14/09				Teleconference – FDA and Gen-Probe
08/13/09		FDA	Gen-Probe	Email
08/13/09		Gen-Probe	FDA	Email
08/12/09		FDA	Gen-Probe	Email
07/31/09 and 08/07/09		FDA	Gen-Probe	Email
08/05/09		Gen-Probe	FDA	Email
08/04/09		FDA	Gen-Probe	Email
07/27/09		FDA	Gen-Probe	Response
07/24/09		Gen-Probe	FDA	Response
07/21/09		Telecon		Teleconference - FDA and Gen-Probe
07/20/09		FDA	Gen-Probe	Email
07/15/09		FDA	Gen-Probe	Email
07/15/09		Gen-Probe	FDA	Document sent
07/14/09		FDA	Gen-Probe	Document sent
07/13/09		Gen-Probe	FDA	Teleconference

PROGENSA® PCA3 Assay
PMA P100033
Pre-IDE # I060180 / I090310
Reverse Chronological Correspondence Index

DATE ORIG	DATE REC'D	TO	FROM	REFERENCE
07/13/09		FDA	Gen-Probe	Teleconference
07/09/09		FDA	Gen-Probe	Email
07/07/09	7/07/09	Gen-Probe	FDA	Comments
07/07/09	7/07/09	Gen-Probe	FDA	Acknowledgement
06/22/09	6/22/09	Gen-Probe	FDA	Comments
06/15/09		FDA	Gen-Probe	Response
06/12/09	6/12/09	Gen-Probe	FDA	Comments
06/11/09		Gen-Probe	FDA	Telephone call
06/02/09		Gen-Probe	FDA	Email
05/27/09		FDA	Gen-Probe	Questions
05/15/09		FDA	Gen-Probe	Questions
05/06/09		FDA	Gen-Probe	Email
05/06/09		Gen-Probe	FDA	Email
05/05/09	5/05/09	Gen-Probe	FDA	Assignment of new pre-IDE number I090310
05/04/09		FDA	Gen-Probe	Document package sent
05/01/09	5/01/09	Gen-Probe	FDA	Email
04/27/09		Gen-Probe	FDA	Document sent
04/27/09		Gen-Probe	FDA	Email
04/24/09		FDA	Gen-Probe	Email
04/17/09		Gen-Probe	FDA	Email
04/13/09		FDA	Gen-Probe	Email
04/03/09		Gen-Probe	FDA	Email
04/01/09		FDA	Gen-Probe	E-mail
03/2609		Gen-Probe	FDA	E-mail
03/05/09		FDA	Gen-Probe	E-mail
02/26/09				Teleconference – FDA and Gen-Probe
11/26/08	12/05/08	Gen-Probe	FDA	Document sent
11/26/08		Gen-Probe	FDA	Fax
11/24/08		FDA	Gen-Probe	Email
10/30/08		Gen-Probe	Gen-Probe	Email
10/14/08		Gen-Probe	Gen-Probe	Email
10/10/08	10/16/08	Gen-Probe	FDA	Letter
10/10/08	10/10/08	Gen-Probe	FDA	Fax

Date	Milestone
08/09/10	Submit PMA to FDA
08/10/10	FDA reference number assigned to PMA: P100033
08/24/10	Formal letter received which assigned the PMA reference number (P100033)
09/17/10	Receipt of PMA filing memo
09/22/10	Receipt of QS Deficiency Letter
10/12/10	Initiation of BIMO inspection at first site
10/13/10	Initiation of BIMO inspection at testing site
10/18/10	Completion of BIMO inspection at testing site
10/20/10	Completion of BIMO inspection at collection site
10/22/10	Submit PMA Amendment 1
11/08/10	Submit PMA Amendment 2
11/29/10	Initiation of BIMO inspection at Sponsor site (GP)
11/29/10	FDA issues Major Deficiency Letter (stops FDA review clock)
12/02/10	Completion of BIMO inspection at Sponsor site (GP)
12/06/10	Receipt of official Major Deficiency Letter via mail
12/06/10	FDA issues second QS Deficiency Letter
12/08/10	Initiation of BIMO inspection at collection site

Exhibit 18

Date	Milestone
12/10/10	Receipt of second QS Deficiency Letter (draft provided electronically by reviewer)
12/14/10	Completion of BIMO inspection at collection site
12/14/10	Completion of all BIMO inspections
12/27/10	Receipt of official second QS Deficiency Letter via fax
01/04/11	Receipt of official second QS Deficiency Letter via mail
01/21/11	FDA accepts all responses to second QS Deficiency Letter
01/24/11	Submit PMA Amendment 3
02/02/11	FDA teleconference to discuss issues that will be presented at Feb 11, 2011 meeting
02/11/11	Face-to-face meeting with FDA
03/08/11	Initiation of pre-approval inspection
03/10/11	First set of MDL responses sent to FDA (Batch #1)
03/18/11	PAI completed
04/01/11	Second set of MDL responses sent to FDA (Batch #2)
04/07/11	Third set of MDL responses sent to FDA (Batch #3)
04/19/20	Kick-Off meeting
04/21/11	Fourth set of MDL responses sent to FDA (Batch #4)
05/03/11	Confirmed with FDA that advisory panel date is Sept 29, 2011

Date	Milestone
05/13/11	Submit PMA Amendment 4
05/20/11	Fifth set of MDL responses sent to FDA (Batch #5)
05/31/11	Sixth (final) set of MDL responses sent to FDA (Batch #6)
06/10/11	Mini-Mock of Panel Meeting
06/14/11	FDA confirms new panel date: Oct 14, 2011
07/13/11	Mock #1 of Panel Meeting
08/04/11	Mock #2 of Panel Meeting
08/10/11	Submit PMA Amendment 5
08/19/11	Federal Register Notice of Advisory Panel Meeting - Oct 14, 2011
08/23/11	Batch #7 responses sent to FDA
08/24/11	FDA postpones Advisory Panel Meeting
09/02/11	Batch #8 responses sent to FDA
09/07/11	Federal Register Notice of Advisory Panel Meeting Postponement
09/11/11	Batch #9 responses sent to FDA-Final set of MDL responses
09/21/11	FDA teleconference
10/07/11	Submit PMA Amendment 6
10/11/11	PMA Amendment 6 filed (re-starts the clock)

Date	Milestone
10/14/11	FDA teleconference to discuss Batch #7 feedback
11/04/11	FDA notifies GP that a panel meeting is not required for the PMA
12/19/11	Receipt of the Approvable letter
01/24/12	Final labeling submitted to FDA via email.
01/25/12	Submit PMA Amendment 7
02/15/12	PMA Approval